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Lactate and Acrylate Metabolism by *Megasphaera elsdenii* under Batch and Steady-State Conditions

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The growth of *Megasphaera elsdenii* on lactate with acrylate and acrylate analogues was studied under batch and steady-state conditions. Under batch conditions, lactate was converted to acetate and propionate, and acrylate was converted into propionate. Acrylate analogues 2-methyl propenoate and 3-butenoate containing a terminal double bond were similarly converted into their respective saturated acids (isobutyrate and butyrate), while crotonate and lactate analogues 3-hydroxybutyrate and (*R*)-2-hydroxybutyrate were not metabolized. Under carbon-limited steady-state conditions, lactate was converted to acetate and butyrate with no propionate formed. As the acrylate concentration in the feed was increased, butyrate and hydrogen formation decreased and propionate was increasingly generated, while the calculated ATP yield was unchanged. *M. elsdenii* metabolism differs substantially under batch and steady-state conditions. The results support the conclusion that propionate is not formed during lactate-limited steady-state growth because of the absence of this substrate to drive the formation of lactyl coenzyme A (CoA) via propionyl-CoA transferase. Acrylate and acrylate analogues are reduced under both batch and steady-state growth conditions after first being converted to thioesters via propionyl-CoA transferase. Our findings demonstrate the central role that CoA transferase activity plays in the utilization of acids by *M. elsdenii* and allows us to propose a modified acrylate pathway for *M. elsdenii*.

Megasphaera elsdenii is an ecologically important rumen bacterium whose genome has recently been sequenced (20) and which metabolizes DL-lactate principally to propionate and acetate (5, 7, 19). Lactate conversion to propionate occurs via the acrylate pathway with acrylyl coenzyme A (CoA) serving as an intermediate (32), a pathway also used by several other organisms, including *Clostridium propionicum*. The dead-end reduction of lactate to propionate allows the cell to balance the anaerobic oxidation of lactate to acetate and carbon dioxide (16), steps which appear to be the primary means of ATP generation (26). *M. elsdenii* also produces butyrate, and several strains also accumulate longer-chain fatty acids from the fermentation of lactate (9). The generation of butyrate from lactate relies on the presence of acetate, and *M. elsdenii* also has the flexibility to generate hydrogen from reduced ferredoxin as another means to balance redox (11). While *M. elsdenii* continues to be of great interest as a member of the rumen microbial community (21, 31), the organism and its enzymes also have potential biotechnological applications (28).

Key steps in the metabolic pathway of *M. elsdenii* reduction of lactate are mediated by propionyl-CoA transferase (24), lactyl-CoA dehydratase (2, 14, 17), and acrylyl-CoA reductase (3, 10). Propionyl-CoA transferase (EC 2.8.3.1; systematic name, acetyl-CoA:propionate-CoA-transferase) is typically implicated in the interconversion of propionate/propionyl-CoA and DL-lactate/DL-lactyl-CoA (26). However, this enzyme is a general CoA-transferase which can mediate other thioester exchanges, including acetate/acetyl-CoA and butyrate/butyryl-CoA (24). Lactyl-CoA dehydratase (EC 4.2.1.54) has relatively low specific activity in the dehydration of lactyl-CoA to acrylyl-CoA (26) and can also mediate the reverse hydration (17). Acrylyl-CoA appears not to accumulate appreciably because of the abundance of acrylyl-CoA reductase (EC 1.3.1.X) in the soluble protein fraction and its very low K_m (10), enabling the propionyl-CoA that is generated to achieve an equilibrium with propionate and other acid-thioester pairs via the transferase. Although the enzyme propionyl-CoA transferase from *M. elsdenii* may be involved in various thioester/acid interconversions, the effect of other substrates on microbial physiology has not been established.

Previous studies with pure cultures of *M. elsdenii* using lactate as a carbon source have primarily investigated cells grown in batch culture. Under batch conditions, lactate is present in excess for essentially the duration of growth. Washed cell suspensions of *M. elsdenii* also ferment lactate and acrylate to yield acetate and propionate as products (18). *M. elsdenii* is similarly able to grow on sugars like glucose and sucrose, although from these substrates butyrate and not propionate is the principal end product (13, 19). Although continuous cultures of *M. elsdenii* have been completed (23), these studies focused on maintenance and energetics and not on product formation. With *Clostridium neopropionicum*, which also uses the acrylate pathway, Seeliger et al. (27) reported no butyrate formation and observed no significant difference in the proportion of propionate and acetate between continuous and batch processes on lactate. Because of its ability to metabolize acrylate and the growing use of *M. elsdenii* enzymes for biotechnological applications, the goals of this study were to investigate the metabolism of acrylate under both batch and steady-state continuous culture.

MATERIALS AND METHODS

Strain and media. *M. elsdenii* strain ATCC 17753 was used in this study. The medium used for serum bottle/culture tube studies contained (per liter) 7.7 g DL-lactate (86 mM), 3.0 g peptone, 4.0 g yeast extract, 0.3 g

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TABLE 1 Molar product yields of *M. elsdenii* growth on lactate and lactate-unsaturated acid mixtures in serum bottles

	Amt (mol/mol lactate)						
	Consumption			Generation			
Substrate(s)[a]	Acrylate	2-MP	3-BA	Acetate	Propionate	Butyrate	Isobutyrate
Lactate (35 mM)				0.38	0.47	0.03	0
Lactate (35 mM) plus:							
Acrylate (5 mM)	0.74			0.67	0.85	0.01	0
2-MP (5 mM)		0.55		0.69	0.15	0.03	0.46
3-BA (5 mM)			1.78	1.06	0.05	1.53	0

[a] 2-MP, 2-methyl propenoate; 3-BA, 3-butenoate.

cysteine-HCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.018 g $FeSO_4 \cdot 7H_2O$, 5.0 ml of 1 mol l^{-1} potassium phosphate, pH 7.0, 2.5 ml saturated $CaSO_4$, and 0.1 mg resazurin. The gas atmosphere was 100% N_2. For batch and steady-state fermentations in the bioreactor, the medium contained (per liter) 3.15 g DL-lactate (35 mM) and 0 to 2.5 g acrylate (0 to 35 mM), 0.6 g yeast extract, 0.5 g cysteine-HCl, 40 ml mineral solution, and 1 ml vitamin solution. The mineral solution contained (per liter) 0.20 g $CaCl_2$, 0.20 g $MgSO_4 \cdot 7H_2O$, 1.00 g K_2HPO_4, 1.00 g KH_2PO_4, 10.0 g $NaHCO_3$, and 2.00 g NaCl. The vitamin solution contained (per liter) 0.5 mg biotin, 20 mg pyridoxine, and 20 mg calcium pantothenate. The pH of the medium for bioreactor studies was adjusted to 6.5 using NaOH.

Growth conditions. *M. elsdenii* was routinely cultured in 150-ml serum bottles containing 100 ml of medium. To study the effect of acrylate on the growth rate, cultures were first grown without acrylate for about 15 h (optical density [OD], ~0.5) in serum bottles, and then 0.5 ml of this culture was used to inoculate an identical medium in a 10-ml tube which could be directly inserted into the spectrophotometer. When this second culture attained an OD of 0.1, acrylate (0 to 35 mM) was added anaerobically, with negligible volume change. Several chemical analogues of acrylate and lactate were similarly examined by addition of 5 mM when the culture attained an OD of 0.1: 2-methyl propenoate (2-MP), 3-butenoate (3-BA), *trans*-2-butenoate (crotonate), 3-hydroxybutyrate, and (*R*)-2-hydroxybutyrate. For the measurement of growth rate, samples were taken every 15 min for 2 h and also stored at −20°C for subsequent chromatographic analysis.

Batch studies using a 1.0-liter medium volume were conducted in a 2.5-liter bioreactor (Bioflo III; New Brunswick Scientific Co. Edison, NJ). In this case, 50 ml of culture initially grown in a serum bottle was transferred to 950 ml bioreactor medium. Nitrogen was sparged at 0.1 liter/min throughout the process, and the agitation was at 150 rpm. The temperature was maintained at 37°C, and the pH was controlled at 6.5 with 15% HCl.

Continuous, steady-state fermentations of a 1.0-liter volume in a 2.5-liter bioreactor operated at a dilution rate of 0.125 h^{-1} as carbon-limited chemostats after initiation in the batch mode as described above. A steady-state condition was assumed after four residence times at which time the CO_2 concentration in the effluent gas remained unchanged. The temperature was maintained at 37°C, and the pH was controlled at 6.5 using 15% HCl.

Analytical methods. The OD at 600 nm (UV-650 spectrophotometer; Beckman Instruments, San Jose, CA) was routinely used to monitor cell growth. Cell dry weight was measured by centrifuging/washing a 40- to 100-ml sample three times in deionized water and drying for 24 h at 60°C. The concentration of carbon dioxide in the off-gas was measured using a gas analyzer (Innova 1313 gas monitor; Lumasense Technologies, Ballerup, Denmark). The hydrogen in the effluent was quantified using gas chromatography (6850 Network GC system; Agilent Technologies) as previously described (25). Concentrations of soluble organic compounds were determined by high-performance liquid chromatography using a refractive index detector, comparing unique retention times and integrated areas to those of pure standards for each analyte at five concentrations (0.5 to 10 mM) (6).

Enzyme assays. Propionyl-CoA transferase was measured at 25°C in 1.0 ml containing (final concentrations) 100 mM potassium phosphate, pH 7.0, 200 mM sodium acetate, 1.0 mM oxaloacetate, 1.0 mM 5,3′-dithiobis(2-nitrobenzoate), 20 μg/ml citrate synthase, and 0.1 mM propionyl-CoA (Sigma-Aldrich Chemical Co., St. Louis, MO) (4). The reaction was initiated by addition of the cell extract, and the increase of absorbance was followed at 405 nm ($\Delta\varepsilon = 13.6$ liters/mmol · cm). Acetate kinase activity was measured colorimetrically at 540 nm by the reaction of acetate phosphate with ferric iron (22). The components were 100 mM Tris-HCl buffer (pH 7.4), 10 mM ATP, 10 mM $MgCl_2$, 700 mM hydroxylamine, and 800 mM potassium acetate. The reaction mixture was maintained at 37°C for 5 min, and then cell extract was added to initiate the reaction and 10% trichloroacetic acid was added to terminate the reaction. Butyrate kinase was measured as described previously (8). For these enzymes, 1 U of activity is the amount of enzyme which generates 1 μmol of product per minute.

RESULTS

Utilization of unsaturated short-chain acids. *M. elsdenii* is a rumen bacterium capable of growing on the DL-lactate which accumulates as a result of a high fermentable sugar diet (5). *M. elsdenii* (12), as well as *Clostridium propionicum* (15) and several other organisms, use the acrylyl-CoA pathway and metabolize lactate and acrylate. We first compared the *M. elsdenii* metabolism of lactate and acrylate under batch conditions in serum bottles and also investigated whether this organism metabolized analogues of acrylate and lactate. Specifically, we examined the unsaturated acids 2-MP, 3-BA, and crotonate and the hydroxyl acids 3-hydroxybutyrate and (*R*)-2-hydroxybutyrate. Of all these organic acids, only lactate supported growth as the sole carbon source (data not shown). When growth was initiated on lactate and one of these compounds was added, several of these organic acids were metabolized, and Table 1 shows their mean product yields and relative substrate consumptions. *M. elsdenii* did not utilize crotonate, 3-hydroxybutyrate, or (*R*)-2-hydroxybutyrate added to a lactate fermentation, although none of these compounds affected continued growth on lactate (data not shown).

When lactate was the sole carbon source in serum bottles, this carbon source partitioned between acetate and propionate at a ratio of about 4:5. Assuming that CO_2 was generated by the formation of acetate and 2 mol of acetyl-CoA was used to generate butyrate, the carbon recovery was 91%. When 3-carbon acrylate was added to the fermentation mixture, the extent of acrylate consumption was 74% on a molar basis of the extent of lactate consumption, and the molar yield of acetate and propionate was again in a ratio of about 4:5 with 89% carbon recovery. The branched acid 2-MP was consumed to an extent of 55% of the lactate consumed, and the branched saturated acid isobutyrate was observed as a product (87% recovery). About 1.78 times as much 3-BA as

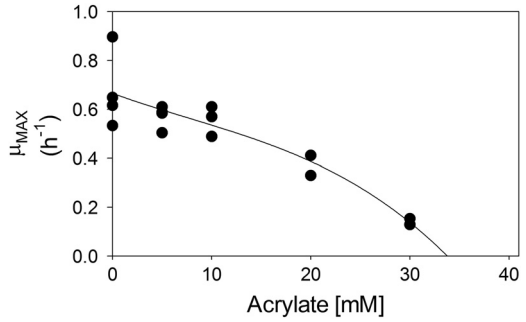

FIG 1 Maximum specific growth rate (●) of *M. elsdenii* ATCC 17753 in lactate medium containing different initial concentrations of acrylate.

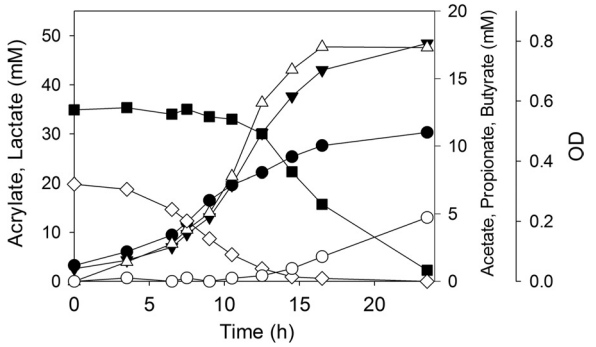

FIG 3 Controlled, batch growth of *M. elsdenii* on lactate (■) and acrylate (◇) generating acetate (▼), propionate (△), and butyrate (○). Cell growth is indicated by OD (●).

lactate was consumed on a molar basis after this unsaturated acid was added to the growing culture, and butyrate was observed to increase proportionately (93% recovery, assuming that newly observed butyrate is derived from 3-BA).

Batch growth on lactate and acrylate. In preparation for batch studies using both lactate and acrylate, we next determined the growth rate of *M. elsdenii* on lactate in the presence of acrylate in serum bottles. We found the maximum specific growth rate (μ_{max}) in the absence of acrylate to average 0.62 h^{-1}. The μ_{max} declined only slightly with increasing acrylate concentrations to 10 mM (Fig. 1). Above 10 mM acrylate, μ_{max} declined more substantially, resulting in a growth rate of less than 0.15 h^{-1} at 30 mM acrylate. No growth was observed in the presence of 35 mM or greater acrylate.

The growth and product distribution of *M. elsdenii* on lactate with and without added acrylate were compared in anaerobic batch cultures in a controlled bioreactor (about 55 mM total acid concentration). In the absence of acrylate, the final molar proportion of acetate-propionate-butyrate generated was 10:8.0:4.7 (Fig. 2). Similar to previous observations, butyrate accumulated at a greater rate late in the fermentation. Considering that CO_2 was generated by the formation of acetyl-CoA (and acetate) and 2 mol of acetyl-CoA was used to generate butyrate, the carbon recovery for the process was 93%, with 66% of the lactate flux partitioning to acetate/butyrate and 27% of the lactate flux partitioning to propionate. Assuming that 1 mol of ATP was generated with each mole of butyrate and acetate formed, the cell yield on ATP (Y_{ATP}) was measured to be 12.5 g cells/mol ATP.

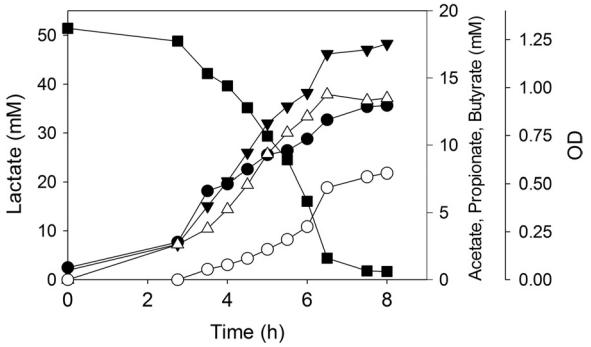

FIG 2 Controlled, batch growth of *M. elsdenii* on lactate (■) alone generating acetate (▼), propionate (△), and butyrate (○). Cell growth is indicated by OD (●).

In the presence of 20 mM acrylate in the bioreactor, the batch growth of *M. elsdenii* was slowed as expected due to reduced tolerance to acrylate (Fig. 3). Nevertheless, the rate of consumption of acrylate was initially much higher than the rate of lactate consumption. For example, during the first 12.5 h of growth, 4.9 mM lactate and 17.2 mM acrylate had been consumed, while 10.0 mM acetate, 13.2 mM propionate, and 0.4 mM butyrate had been generated. The carbon recovery for this portion of the process was 109%, with 49% of the lactate/acrylate partitioning to acetate/butyrate and 60% partitioning to propionate. The Y_{ATP} during this portion was 12.5 g cells/mol ATP. At between 12.5 h and 23.5 h, the remaining substrates were consumed (27.7 mM lactate and 2.6 mM acrylate), while 6.6 mM acetate, 4.1 mM propionate, 4.3 mM butyrate, and only an additional 0.05 g/liter cells were generated.

Steady-state growth on lactate and acrylate. We next performed several carbon-limited chemostats at a growth rate of 0.125 h^{-1} to compare steady-state metabolism in the presence of increasing acrylate concentrations in the feed (0, 10, and 20 mM). Because *M. elsdenii* showed a maximum growth rate far above a dilution rate of 0.125 h^{-1} with 20 mM acrylate (Fig. 1) and because acrylate was readily metabolized in batch culture (Fig. 3), we anticipated that acrylate would be depleted under carbon-limited steady-state conditions, which was indeed the case (steady-state concentrations, about 1 mM). In addition to measuring organic acid products, we measured hydrogen and CO_2 in the effluent gas. We were unable to attain a steady state when the acrylate concentration was above 20 mM in the presence of 35 mM lactate.

With lactate as the sole carbon source (i.e., no acrylate), essentially no propionate formed in duplicate experiments under steady-state conditions. Instead, butyrate was the primary acid product. We observed a mean specific lactate consumption rate (q_{LAC}) of 39.2 mmol/g · h, with products formed at a rate of 4.6 mmol acetate/g · h, <0.5 mmol propionate/g · h, 10.4 mmol butyrate/g · h, 45.6 mmol CO_2/g · h, and 31.1 mmol H_2/g · h (88% carbon recovery). Assuming again that acetate and butyrate formation generated ATP, Y_{ATP} was 8.3 g cells/mol ATP.

As expected, both lactate and acrylate were consumed in the steady-state processes containing both substrates, and increasing the proportion of acrylate from 0 mM to 20 mM in the feed therefore increased the fraction of acrylate consumed relative to the total organic acids. In order to quantify the effect of an increasing acrylate proportion in the mixture, we defined the molar acrylate

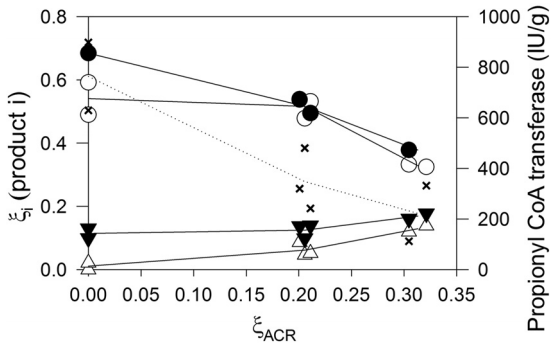

FIG 4 Molar fraction of the total substrate flux (lactate plus acrylate) to each product under carbon-limited steady-state conditions (chemostat) versus the fraction of acrylate consumed (ξ_{ACR}) in an acrylate and lactate mixture. Fraction butyrate (ξ_{BUT}, ○) = 2 × number of moles of butyrate generated/(number of moles of lactate consumed + number of moles of acrylate consumed); fraction acetate (ξ_{ACE}, ▼) = number of moles of acetate generated/(number of moles of lactate consumed + number of moles of acrylate consumed); fraction propionate (ξ_{PRO}, △) = number of moles of propionate generated/(number of moles of lactate consumed + number of moles of acrylate consumed); fraction hydrogen (ξ_{H2}, ●) = number of moles of propionate generated/(number of moles of lactate consumed + number of moles of acrylate consumed). The specific activity (IU/mg dry cell weight) of propionyl-CoA transferase (✖) is also shown.

fraction consumed (ξ_{ACR}) to be $q_{ACR}/(q_{ACR} + q_{LAC})$, where q is the specific rate of substrate consumption, LAC is lactate, and $\xi_{ACR} + \xi_{LAC}$ is equal to 1. Then, the fraction of that total molar substrate flux which partitioned toward a particular product I (ξ_I) was calculated. For example, considering that a mole of acetate (ACE) is generated from 1 mol of lactate or acrylate, the fractional flux of lactate or acrylate to form acetate is $\xi_{ACE} = q_{ACE}/(q_{ACR} + q_{LAC})$. Similarly, 1 mol of butyrate (BUT) requires 2 mol of either substrate so that $\xi_{BUT} = 2 \times q_{BUT}/(q_{ACR} + q_{LAC})$.

Increasing the proportion of acrylate consumed under steady-state conditions from an acrylate-lactate mixture (ξ_{ACR}) increased propionate and acetate formation (ξ_{LAC}, ξ_{ACE}) and decreased butyrate formation (ξ_{BUT}) (Fig. 4). For example and as noted above, in the absence of acrylate, about 12% of the lactate was converted to acetate and 54% of the lactate was converted to butyrate. When acrylate comprised about one-third of the total substrate consumed, 17% of the two substrates was converted to acetate, 33% of these substrates was converted to butyrate, and 13% was converted to propionate (with carbon recoveries of 70 to 92%).

Increasing the proportion of acrylate consumed under steady-state conditions decreased the fraction of H_2 flux ($\xi_{H2} = q_{H2}/(q_{ACR} + q_{LAC})$). The decrease in H_2 flux was more than could be accounted for by the sum of the acetate and butyrate flux. For example, in the absence of acrylate, the hydrogen flux (q_{H2} = 31.1 mmol/g.h) was close to the value obtained for the sum $q_{ACE} + (2 \times q_{BUT})$ (28.1 mmol/g · h), suggesting that one hydrogen was generated for each acetyl-CoA formed. However, in the presence of 20 mM acrylate in the feed, q_{H2} (23.6 mmol/g · h) was lower than $q_{ACE} + (2 \times q_{BUT})$ (30.7 mmol/g · h), suggesting that some other reduced product was generated in conjunction with the formation of acetyl-CoA. Although *M. elsdenii* is known to generate valerate and caproate under certain conditions (19), we did not detect these higher-molecular-weight acids in this open system. The amount of acrylate consumed was greater than this difference between acetyl-CoA and hydrogen generation. Again, considering that 1 mol of ATP was generated for each acetate and butyrate produced during the chemostat experiments containing acrylate, the average Y_{ATP} was 10.0 g cells/mol ATP when 10 mM acrylate was present in the feed and 9.0 g cells/mol ATP when 20 mM acrylate was present in the feed. Expressed in terms of the amount of substrates consumed, 0.33 to 0.40 mol ATP was generated per mol of total acids (lactate plus acrylate) consumed, with no apparent trend as the fraction of acrylate in the feed increased.

Increasing the proportion of acrylate consumed decreased the specific activity of propionyl-CoA transferase. No trend for the activity of acetate kinase was observed with increasing amount of acrylate consumed, which was generally measured to be 1.0 to 2.5 IU/g cell dry weight (data not shown). We failed to detect butyrate kinase activity in any of the batch or chemostat processes (data not shown).

DISCUSSION

Megasphaera elsdenii oxidizes DL-lactate to acetate, with ATP generated, while it simultaneously reduces DL-lactate to propionate via acrylyl-CoA using the acrylate pathway. *M. elsdenii* appears to have an NAD-dependent D-lactate dehydrogenase and a lactate racemase (13). In our experiments, we observed complete utilization of DL-lactate with no apparent diauxic growth (Fig. 2). Although acrylate utilization by *M. elsdenii* and other organisms using the acrylyl-CoA pathway has been established, the details of acrylate assimilation have not been clarified. Most investigators propose direct assimilation to acrylyl-CoA via an acrylyl-CoA synthetase (12, 29). Our observations cast doubt on the presence of an acrylyl-CoA synthetase, instead pointing to the assimilation of acrylate and analogues via the general CoA transferase. First, the assimilation of acrylate (and unsaturated analogues 2-MP/3-BA) to propionate (and isobutyrate/butyrate) suggests either their direct reduction to the saturated acids or reduction via the intermediates acrylyl-CoA (and 2-MP-CoA/3-BA-CoA). However, we did not observe butyrate formation from the unsaturated analogue crotonate, and no direct reductase acting on acids has been identified in *M. elsdenii*, supporting the indirect reduction of the unsaturated acids via the thioester derivatives. Second, the specific analogues metabolized by *M. elsdenii* in our studies are consistent with previous observations which indicate that propionyl-CoA transferase is active with acrylyl-CoA (30), butyryl-CoA, and isobutyryl-CoA but not crotonyl-CoA (24). Third, if acrylate were assimilated through an acrylyl-CoA synthetase, an ATP requirement for this thioester bond formation would be expected. However, in the batch process (Fig. 3), acrylate utilization was initially faster than lactate utilization, while under steady-state conditions, increasing the fraction of acrylate consumed did not affect ATP yield, results which would not be expected with an ATP-utilizing CoA synthetase. Collectively, our results support the hypothesis that the first step in the utilization of these metabolized acids is through the propionyl-CoA transferase or via some other unknown ATP-conserving enzyme, such as other enzymes with CoA transferase activity.

Interestingly, *M. elsdenii* reduced branched and straight-chain acids which were unsaturated at a terminal carbon (e.g., 2-MP) but not unsaturated acids in which the double bond occurred between intermolecular carbons (e.g., crotonate) or either of two lactate analogues. The inability to metabolize crotonate and 3-hydroxybutyrate is surprising, considering that the thioesters of these acids are presumably intermediates in the butyrate synthesis pathway (33). As noted above, the inability of *M. elsdenii* to metabolize crotonate may simply be a

specific consequence of propionyl-CoA transferase not donating a CoA to this acid, as previously observed (24). To our knowledge, the ability of propionyl-CoA transferase to donate a CoA to 3-hydroxybutyrate and (*R*)-2-hydroxybutyrate has not been examined. Our results do not exclude the possibility that propionyl-CoA transferase is indeed active toward these two hydroxylated acids and that the ultimate inability of *M. elsdenii* to assimilate them is instead due to the specificity of lactyl-CoA dehydratase toward lactyl-CoA.

Using lactate as the sole carbon source in the absence of acrylate, under batch conditions, *M. elsdenii* principally generates propionate (and acetate), whereas under carbon-limited steady-state conditions, essentially no propionate is generated. These significant results reinforce a key difference between a batch process and a carbon-limited chemostat. Under batch conditions, lactate is present in excess through the duration of growth. The relatively high concentration of lactate compared to the concentrations of other acids can provide a driving force for that acid to be converted into its thioester via the propionyl-CoA transferase. Once lactyl-CoA forms in the presence of excess lactate, the organism is committed to propionyl-CoA formation. Although several thioesters could serve as the CoA donor for the initial conversion of lactate to lactyl-CoA, propionyl-CoA is likely the primary donor, as no other conversion appears to be available to prevent propionyl-CoA from accumulating. Acetyl-CoA, another potential CoA donor (which would result in no ATP formation), serves as a substrate for other enzymes, such as acetate kinase and acetoacetyl-CoA thioesterase. The continued demand for a redox balance provides a continuous supply of propionyl-CoA from acrylyl-CoA to ensure the accumulation of propionate.

In contrast, under chemostat carbon-limited conditions, lactate is essentially absent in the medium (despite it being continuously consumed) and would similarly be limiting intracellularly. With minimal lactate present, little driving force would exist to generate lactyl-CoA and, subsequently, propionyl-CoA and propionate. Our results are conclusive: under steady-state conditions in the absence of lactate, the lactate flux was exclusively directed toward acetate or butyrate and the highest H_2 production. This equilibrium model for propionyl-CoA transferase also provides a simple explanation for the absence of propionate formation when glucose serves as the sole carbon source (12): when glucose is the sole carbon source, the concentration of lactate may never become great enough to drive the formation of lactyl-CoA with the simultaneous conversion of propionyl-CoA to propionate. Interestingly, our results suggest that lactyl-CoA dehydratase, presumably necessary under batch growth on lactate, becomes dispensable under steady-state conditions or in the presence of acrylate.

Under steady-state conditions, the propionyl-CoA transferase activity decreased with increasing acrylate concentration in the feed. At the highest concentration of acrylate (20 mM), the enzyme activity measured was about 200 IU/g or 12 mmol/g · h (Fig. 4), equivalent to a total substrate interconversion of 24 mmol/g · h. At this feed concentration, the specific rates of consumption/formation were nominally 30 mmol lactate/g · h, 15 mmol acrylate/g · h, 7 mmol propionate/g · h, 10 mmol acetate/g · h, and 10 mmol butyrate/g · h. Although the sum of these rates might appear to exceed the available enzyme activity, as proposed above, lactate may be converted exclusively to acetate and butyrate and may not itself serve as a substrate for the transferase. The sum of the rates of formation for propionate and butyrate (which simultaneously utilize acrylate and acetate) is close to the measured enzyme activity. Furthermore, the enzyme activity was measured specifically by coupling the transfer of propionyl-CoA to acetate and does not account for the differential affinities between other acids and thioesters (24, 26). Although the assay would be expected to capture the activities of other CoA-transferring enzymes capable of donating CoA to acetate, our results do not preclude the possibility of multiple CoA-transferring enzymes existing in *M. elsdenii*.

So, why is butyrate instead of acetate the principal product from lactate under steady-state conditions? Formation of acetate and butyrate from lactate can be compared by considering the stoichiometric equations for each conversion. For acetate formation, the sequential action of lactate dehydrogenase, pyruvate ferredoxin oxidoreductase, phosphotransacetylase, and acetate kinase yields

$$\text{lactate} + P_i + \text{ADP} + \text{NAD} + \text{Fd} \rightarrow \text{acetate} + \text{NADH} + \text{FdH}_2 + CO_2 + \text{ATP} \quad (1)$$

where Fd denotes ferredoxin. Butyrate formation requires 2 mol of acetyl-CoA for the formation of 1 mol of acetoacetyl-CoA and acetoacetyl-CoA reduced (using 2 mol NADH) to butyryl-CoA, which is converted to butyrate via a transferase with a CoA acceptor such as acetate (no butyrate kinase activity was detected). The equation thus becomes

$$2\text{lactate} + P_i + \text{ADP} + 2\text{Fd} \rightarrow \text{butyrate} + \text{ATP} + H_2O + 2CO_2 + 2\text{FdH}_2 \quad (2)$$

A comparison of Equations 1 and 2 demonstrates that the ATP yield from acetate formation is 1 mol ATP/mol lactate consumed, whereas the ATP yield from butyrate formation is 0.5 mol ATP/mol lactate consumed. Both butyrate and acetate formation generate 1 mol ferredoxin H_2/mol lactate consumed. However, the formation of acetate generates 1 mol NADH/mol lactate consumed, whereas the formation of butyrate generates no NADH. In other words, the formation of butyrate generates fewer electrons from lactate than the formation of acetate. Thus, formation of butyrate facilitates the redox balance. Since *M. elsdenii* has the means to generate H_2 from reduced ferredoxin (1), H_2 generation probably allows the cell to attain a balanced redox in the absence of lactate reduction to propionate. Interestingly, the conversion of acetate and acetyl-CoA to butyrate provides the cells with a means of consuming NADH without any ATP utilization:

$$\text{acetate} + \text{acetyl-CoA} + 2\text{NADH} \rightarrow \text{butyrate} + \text{HCoA} + 2\text{NAD} \quad (3)$$

Under steady-state or batch conditions, increasing the proportion of acrylate in the medium increased the proportion of propionate generated. Acrylate has been demonstrated to serve as a CoA acceptor in propionyl-CoA transferase (26), and the low K_m of acrylyl-CoA reductase (10) would likely prevent accumulation of acrylyl-CoA. Our observation of acrylate consumption being faster than lactate consumption is consistent with the higher activity of acrylyl-CoA reductase than that of lactyl-CoA dehydratase. The presence of acrylate appears to provide cells with a means to bypass the comparatively slow lactyl-CoA dehydratase to balance overall redox.

Based on our observations regarding acrylate consumption via propionyl-CoA transferase instead of a thioesterase, we propose a modification of previously suggested pathways of acrylate metabolism (Fig. 5). We furthermore propose that analogues of acrylate follow a path analogous to that of the respective thioester by pro-

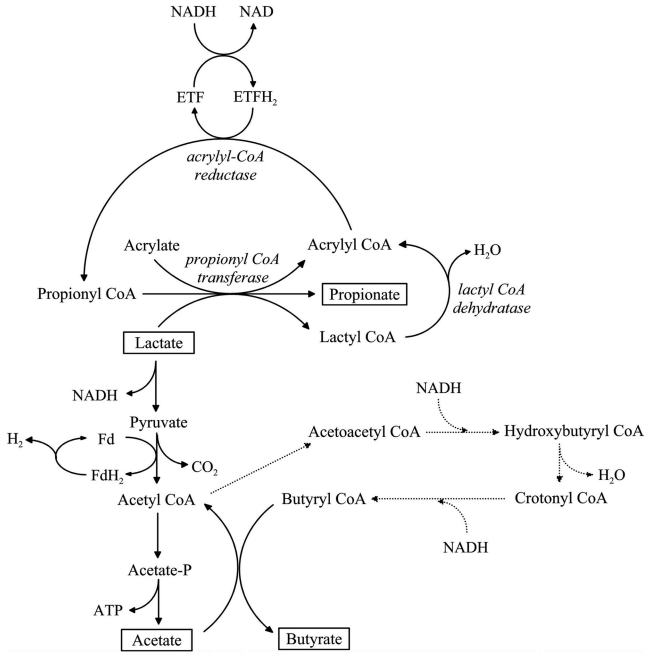

FIG 5 Proposed pathway for metabolism of acrylate and lactate into propionate, acetate, and butyrate by *M. elsdenii*. This pathway is extended and modified from previous models (10, 12, 29) to show acrylate consumption via propionyl-CoA transferase and the absence of butyrate kinase.

pionyl-CoA transferase before being reduced (by acrylyl-CoA reductase, reduced ferredoxin, or another reductase). Washed cell suspensions of *M. elsdenii* have been known to convert lactate and acrylate into pyruvate and propionate, and in the presence of phosphate molecules, the pyruvate generated is further converted to acetate (18). Carbon labeling studies showed that all the pyruvate and acetate generated came from lactate, while the propionate was obtained from acrylate (18). Our results do not determine specifically whether a portion of the acrylyl-CoA is hydrated to lactyl-CoA and even potentially metabolized to acetate/acetyl-CoA but do demonstrate that acrylate relieves the formation of the hydrogen and butyrate.

In summary, the metabolism of organic acids by *M. elsdenii* appears to be controlled by the equilibrium between acids and their thioesters mediated by propionyl-CoA transferase and possibly other transferases. Growth on lactate-limited continuous cultures, such as that previously observed in glucose batch cultures in which lactate was similarly absent, results in a mixture of acetate and butyrate. In contrast, growth in the presence of excess lactate under batch conditions results in a mixture of propionate and acetate. Acrylate and other metabolizable analogues provide cells with a route to bypass the lactyl-CoA dehydratase to form propionate and reduced analogues and thereby another means of attaining a redox balance.

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