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Role of formate and hydrogen in the degradation of propionate and butyrate by defined suspended cocultures of acetogenic and methanogenic bacteria

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Abstract

The butyrate-degrading *Syntrophospora bryantii* degrades butyrate and a propionate-degrading strain (MPOB) degrades propionate in coculture with the hydrogen- and formate-utilizing *Methanospirillum hungatii* or *Methanobacterium formicicum*. However, the substrates are not degraded in constructed cocultures with two *Methanobrevibacter arboriphilus* strains which are only able to consume hydrogen. Pure cultures of the acetogenic bacteria form both hydrogen and formate during butyrate oxidation with pentenoate as electron acceptor and during propionate oxidation with fumarate as electron acceptor. Using the highest hydrogen and formate levels which can be reached by the acetogens and the lowest hydrogen and formate levels which can be maintained by the methanogens it appeared that the calculated formate diffusion rates are about 100 times higher than the calculated hydrogen diffusion rates.

Introduction

Butyrate and propionate are two important intermediates in the mineralization of organic matter under methanogenic conditions. These fatty acids are degraded by syntrophic consortia of acetogenic bacteria and methanogenic bacteria (Schink 1992; Stams 1994). Complete mineralization of butyrate and propionate into methane and CO_2 requires three different types of bacteria. Acetogenic bacteria oxidize butyrate and propionate into acetate and H_2 and/or formate (Table 1, equation 1–4). These products are further converted to methane by H_2/formate-utilizing and aceticlastic methanogens (Table 1, equation 5–7).

Because of unfavorable energetics of propionate and butyrate oxidation, the H_2 and/or formate levels have to be kept extremely low; i.e. at low H_2 and formate concentrations the $\Delta G'$ values of butyrate and propionate oxidation become negative. Interspecies H_2 and formate transfer are proposed as possible mechanisms for the syntrophic degradation by anaerobic consortia (Hungate 1967; Wolin 1982; Thiele & Zeikus 1988; Boone et al. 1989). Although both H_2 and formate were detected in the methanogenic cocultures when methanogenesis was inhibited (Thiele & Zeikus, 1988; Dong et al. 1994a), it still is not clear yet whether H_2 or formate transfer is more important. This in particular because many methanogens and also acetogens are able to interconvert H_2/CO_2 and formate (Wu et al. 1993; Bleicher & Winter 1994; Dong & Stams 1995). Recently, we obtained direct evidence that in defined suspended cocultures degrading butyrate and propionate formate transfer might be more important than hydrogen transfer (Dong et al. 1994a; 1994b; Dong & Stams 1995). Most important results of these investigations are summarized here.

Syntrophic propionate and butyrate-degrading bacteria

Syntrophomonas wolfei, *S. sapovorans* and *Syntrophospora* (former *Clostridium*) *bryantii* and strain SF-1 are anaerobic acetogenic bacteria which degrade butyrate in coculture with methanogens (McInerney et al. 1981; Stieb & Schink 1985; Zhao et al. 1990; Roy

Table 1. Reactions involved in the syntrophic degradation of propionate and butyrate in methanogenic environments.

Eq.	Reaction		$\Delta G^{\circ\prime}$ [kJ]
1.	$Butyrate^- + 2\,H_2O$	$\rightarrow 2\,Acetate^- + H^+ + 2\,H_2$	48.1
2.	$Butyrate^- + 2\,HCO_3^-$	$\rightarrow 2\,Acetate^- + H^+ + 2\,HCOO^-$	45.5
3.	$Propionate^- + 3\,H_2O$	$\rightarrow Acetate^- + HCO_3^- + 3\,H_2 + H^+$	76.1
4.	$Propionate^- + 2\,HCO_3^-$	$\rightarrow Acetate^- + 3HCOO^- + H^+$	72
5.	$4\,H_2 + HCO_3^- + H^+$	$\rightarrow CH_4 + 2\,H_2O$	−135.6
6.	$4\,HCOO^- + H_2O + H^+$	$\rightarrow CH_4 + 3\,HCO_3^-$	−130
7.	$CH_3COO^- + H_2O$	$\rightarrow CH_4 + HCO_3^-$	− 31

et al. 1986; Shelton & Tiedje 1984). It was demonstrated that *S. wolfei* and *S. bryantii* oxidize butyrate by β-oxidation via the crotonyl-CoA pathway (Wofford et al. 1986; Schink 1992). Reducing equivalents are released during the oxidations of butyryl-CoA to crotonyl-CoA and of 3-hydroxybutyryl-CoA to acetoacetyl-CoA. These reducing equivalents are disposed of as hydrogen or as formate by H^+ or CO_2 reduction, respectively.

In a similar fashion, *Syntrophobacter wolinii* and other mesophilic propionate oxidizing bacteria including strain MPOB and KoProp1, oxidize propionate to acetate, CO_2 and hydrogen and/or formate (Boone & Bryant 1980; Stams et al. 1993; Dörner 1992). In syntrophic cocultures propionate is degraded via the methylmalonyl-CoA pathway (Houwen et al. 1987; 1990; Koch et al. 1983; Plugge et al. 1993). In this pathway reducing equivalents are formed in the oxidations of succinate to fumarate, malate to oxaloacetate and pyruvate to acetate, respectively. These reducing equivalents ultimately are disposed of as hydrogen or formate.

The described acetogenic bacteria are also able to grow in the absence of methanogens. The butyrate degraders grow by fermentation of crotonate and other unsaturated fatty acids (Beaty & McInerney 1987; Amos & McInerney 1990; Dong et al. 1994a). *S. wolinii* can grow fermentatively on pyruvate, while strain MPOB grows by fermentation of fumarate (Wallrabenstein et al. 1994; Stams et al. 1993). *S. wolinii* and strains MPOB and KoProp1 also are able to oxidize propionate to acetate with sulfate as electron acceptor (Dörner 1992; Wallrabenstein et al. 1994; van Kuijk & Stams 1995).

Table 2. Highest propionate and butyrate degradation rates measured in cocultures of *S. bryantii* and strain MPOB with different methanogens. Data obtained from Dong et al. 1994a, 1994b.

	S. bryantii	strain MPOB
		$mmol.l^{-1}.day^{-1}$
M. hungatii	2.6	0.8
M. hungatii		
M. soehngenii	3.5	1.4
M. formicicum	0.8	0.4
M. formicicum		
M. soehngenii	1.3	n.d.
M. soehngenii	–	–
M. arboriphilus	–	–
M. arboriphilus		
M. soehngenii	–	–

–: no substrate degradation; n.d.: not determined.
M. hungatii and *M. formicicum* are able to grow with hydrogen and formate, while *M. arboriphilus* and *M. soehngenii* grow only with hydrogen and acetate, respectively.

Constructed cocultures

The ability of the acetogens to grow in the absence of methanogens offers the ability to construct defined cocultures with different methanogenic bacteria. In our studies methanogens were used which are able to grow on H_2/CO_2 and on formate, and methanogens which only can grow on H_2/CO_2. The outcome of these experiments was that the butyrate-degrading *Syntrophospora bryantii* degraded butyrate and strain MPOB degraded propionate in coculture with the hydrogen- and formate-utilizing *Methanospirillum*

Table 3. Calculated H_2 and formate flux in cocultures of *M. hungatei* with MPOB and with *S. bryantii* respectively using the Fick's diffusion equation.

	S. bryantii	strain MPOB
Measured hydrogen/formate flux in the coculture based on methane production rates (nmol.min^{-1}. ml^{-1})	3.1	1.7
Cell surface area of the acetogens calculated from the cell sizes and the cell densities (μm^2.ml^{-1})	1.7*10^9	1.8*10^9
Average distance between cells (μm)	9.2	12.4
Highest H_2 level formed by the acetogen (μM)	1.0	0.04
Highest formate level formed by the acetogen (μM)	280	24
Lowest H_2 level reached by *M. hungatii* (μM)	0.01	0.01
Lowest formate level reached by *M. hungatii* (μM)	15	15
Calculated H_2 flux (nmol.min^{-1}. ml^{-1})	50.4	1.1
Calculated formate flux (nmol.min^{-1}. ml^{-1})	4420	117

hungatii and *Methanobacterium formicicum,* but not in constructed cocultures with two *Methanobrevibacter arboriphilus* strains, which are only able to consume hydrogen (Dong et al. 1994a; 1994b). In constructed bicultures of the acetogens with the aceticlastic methanogen *Methanosaeta* (former *Methanothrix*) *soehngenii* butyrate and propionate were also not degraded. However, the butyrate and propionate degradation rates in tricultures with *M. hungatii* and *M. soehngenii* were always higher than in the bicultures in which the *M. soehngenii* was not present (Table 2)

Hydrogen and formate formation in pure cultures

S. bryantii is able to degrade butyrate in pure culture in the presence of pentenoate as electron acceptor (Dong et al. 1994a), and strain MPOB degrades propionate with fumarate as electron acceptor (Stams et al. 1993). By using limiting amounts of electron acceptors the highest levels H_2 and formate produced by *S. bryantii* and strain MPOB were determined (Dong & Stams 1995). The highest levels of hydrogen and formate reached by *S. bryantii* were 171 Pa (1.0 μM) and 280 μM, respectively. For strain MPOB these values were 7 Pa (0.04 μm) and 24 μm, respectively.

Calculated diffusion rates

Microscopical examination revealed that the syntrophic cocultures with strain MPOB and *S. bryantii* grew suspended. By using the highest concentrations of formate and H_2 that can be reached by the two acetogenic bacteria (see above) and the lowest formate and H_2 concentrations that can be reached by methanogens, the fluxes of formate and H_2 between consortia in the syntrophic culture can be calculated using the Fick's diffusion equation:

$$Flux = D.A.\frac{[C]_{acetogen} - [C]_{methanogen}}{d} \quad (1)$$

where D is the diffusion constant, A is the total surface of the acetogen, $[C]_{acetogen} - [C]_{methanogen}$ is the concentration gradient between the acetogen and the methanogen, and d is the diffusion distance. In our calculations diffusion constants of H_2 and formate in water of 4.5*10^{-5} and 0.15*10^{-5} cm^2.sec^{-1}, respectively, were used. The threshold values for H_2 and formate of *M. hungatii* are 2 Pa (0.01 μM) and 15 μM, respectively (Seitz et al. 1988; Schauer et al. 1982). The outcome of these calculations (Table 3) indicates that formate transfer is more important in syntrophic butyrate and propionate degradation than H_2 transfer. This is in accordance with above-mentioned observation that in defined methanogenic cocultures of the two acetogens with the H_2-utilizing *M. arboriphilus* butyrate and propionate are not degraded.

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