Ann Microbiol (2010) 60:1–12
DOI 10.1007/s13213-009-0008-5

REVIEW ARTICLE

CO in methanogenesis

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Received: 22 September 2009 / Accepted: 9 November 2009 / Published online: 2 February 2010

Abstract Although CO is present in methanogenic environments, an understanding of CO metabolism by methanogens has lagged behind other methanogenic substrates and investigations of CO metabolism in non-methanogenic species. This review features studies on the metabolism of CO by methanogens from 1931 to the present. The pathways for CO metabolism of freshwater versus marine species are contrasted and the ecological implications discussed. The biochemistry and role of CO dehydrogenase/acetyl-CoA synthase in the pathway for conversion of acetate to methane and biosynthesis of cell carbon is presented. Finally, a proposal for the role of CO and primitive forms of the CO dehydrogenase/acetyl-CoA synthase in the origin and early evolution of life is discussed.

Keywords Carbon monoxide · Methanogenesis · Acetate · Acetyl-CoA synthase · Ecology · Evolution

Introduction

The decomposition of complex organic matter in diverse anaerobic environments is an essential link in the global carbon cycle (Fig. 1) producing approximately 1 billion metric tons of methane each year. In the cycle, CO_2 is fixed into complex organic matter by photosynthesis that is decomposed primarily by O_2-requiring aerobic microorganisms in oxygenated habitats with release of CO_2 back into the atmosphere. However, a portion of the organic matter is also deposited in diverse O_2-free habitats where anaerobic microbial food chains decompose the organic matter to CH_4 and CO_2 in a process called biomethanation. A portion of the CH_4 is converted back to CO_2 by anaerobic methylotrophs and the remainder escapes into aerobic zones where it is oxidized to CO_2 by O_2-requiring methylotrophs, thereby completing the global carbon cycle.

The biomethanation of organic matter occurs in diverse habitats such as freshwater sediments, rice paddies, sewage digesters, the rumen, the lower intestinal tract of monogastric animals, landfills, hydrothermal vents, coastal marine sediments, and the subsurface (Liu and Whitman 2008). A minimum of three interacting metabolic groups of anaerobes comprise a consortium converting complex organic matter to CO_2 and CH_4 (Fig. 2). The fermentative group I anaerobes decompose complex organic matter to acetate, higher volatile fatty acids, H_2, and CO_2. The H_2-producing acetogenic group II anaerobes decompose the higher volatile fatty acids to acetate, H_2, and CO_2. The group III and IV methanogens convert the metabolic products of the first two groups to CH_4 by two major pathways. At least two-thirds of the CH_4 produced in nature derives from the conversion of the methyl group of acetate by the Group III methanogens. The Group IV methanogens produce approximately one-third by reducing CO_2 with electrons supplied from the oxidation of H_2 or formate. Thus, methanogens are dependent on the first two groups to supply substrates for their growth. The production of H_2 by Groups I and II is thermodynamically unfavorable, requiring the CO_2-reducing methanogenic Group IV to maintain low concentrations of H_2. A variety of other simple compounds serve as minor substrates for methanogenesis. Ethanol and secondary alcohols are used as electron donors for the reduction of CO_2 to CH_4. The methyl groups of methanol, methylamines, and methylated sulfides are also dismutated

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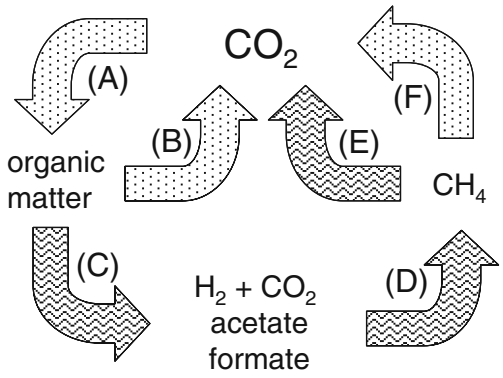

Fig. 1 The global carbon cycle. **a** Fixation of CO_2 into organic matter, **b** aerobic decomposition of organic matter to CO_2, **c** anaerobic decomposition of organic matter to fermentative end products, **d** anaerobic conversion of fermentative end products to CH_4, **e** anaerobic oxidation of CH_4 to CO_2, **f** aerobic oxidation of CH_4 to CO_2

to CO_2 and CH_4. Although CO is present in methanogenic environments (Conrad and Seiler 1980), an understanding of CO metabolism by methanogens has lagged behind other substrates. This review chronicles studies on the metabolism of CO by methanogens from 1931 to the present. For a more comprehensive general understanding of methanogens, the reader is referred to several recent review articles describing the ecology, physiology, and biochemistry of methanogenesis (Ferry 2008; Ferry and Lessner 2008; Liu and Whitman 2008; Oelgeschlager and Rother 2008; Spanheimer and Muller 2008; Thauer et al. 2008).

CO as an energy source

The first recorded report portending the ability of methanogens to metabolize CO appeared in 1931 when it was demonstrated that sewage sludge converts CO to CO_2 and

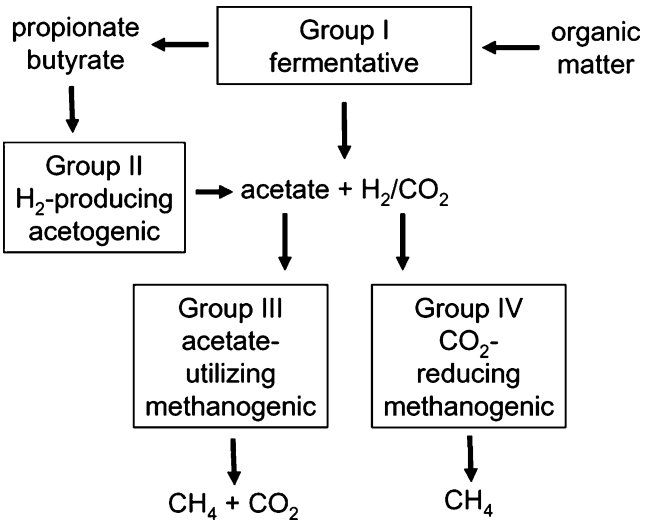

Fig. 2 A freshwater anaerobic microbial food chain. By permission (Ferry 2008)

CH_4 (Fischer et al. 1931). However, the direct conversion of CO by methanogens (Eq. 1) could not be concluded since both H_2 and acetate appeared and disappeared (Fischer et al. 1932) consistent with non-methanogens as the primary anaerobes metabolizing CO to methanogenic substrates (Eqs. 2 and 3).

$$4CO + 2H_2O \rightarrow 3CO_2 + CH_4 \quad (1)$$

$$4CO + 4H_2O \rightarrow 4H_2 + 4CO_2 \quad (2a)$$

$$4H_2 + CO_2 \rightarrow 2H_2O + CH_4 \quad (2b)$$

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad (3a)$$

$$CH_3COOH \rightarrow CO_2 + CH_4 \quad (3b)$$

In 1933, it was reported (Stephenson and Stickland 1933) that a pure culture was able to convert CO to CH_4 by first converting CO to H_2 (Eq. 2ab) that, 14 years later in 1947, was confirmed with resting cell suspensions of a pure culture (Kluyver and Schnellen 1947). Another gap in time had passed before studies on CO metabolism by methanogens resumed in 1977 and 1984 when it was shown that several species remove CO from the gas phase when growing with CO_2 plus H_2 and, for the first time, that two freshwater methanogens are able to grow with CO as the sole energy source, albeit under conditions unable to sustain proliferation in the native environment (Daniels et al. 1977). Only recently, a marine species was shown to grow with CO under conditions suitable for proliferation in the native environment via a novel pathway for methanogenesis consistent with CO-dependent growth in the native environment (Lessner et al. 2006).

Freshwater environments

Methanothermobacter thermoautotrophicus (formerly, *Methanobacterium thermoautotrophicum* strain ΔH) was shown to grow with CO as the sole energy source disproportionating CO according to Eq. 1, although the growth rate was only 1% of that with H_2/CO_2 (Daniels et al. 1977). Cell-free extracts showed CO dehydrogenase activity with coenzyme F_{420} as the electron acceptor. The activity was reversibly inactivated by cyanide and O_2 consistent with an active-site metal center; however, the enzyme was not purified and studied in greater detail.

Methanosarcina barkeri strain MS isolated in 1966 (Bryant and Boone 1987) can be adapted to grow in an

atmosphere of 50% CO as the only carbon and energy source (O'Brien et al. 1984). Net H_2 formation is observed when the headspace CO is greater than 20%. Below this concentration of CO, H_2 is consumed and the rate of CH_4 production increases substantially with an increased growth rate approaching a doubling time of 65 h. *Methanosarcina barkeri* strain MS also produces CH_4 and CO_2 with the growth substrates methanol plus a headspace of 50% CO (O'Brien et al. 1984). However, it was reported that H_2 accumulated and methanol was not metabolized until the CO decreased to below 30% in the headspace, at which point H_2 and methanol was rapidly consumed proportional to an increase in the rate of CH_4 formation and growth. Based on these characteristics, it was concluded that methanogenesis in the freshwater species *M. barkeri* strain MS is inhibited by concentrations of CO greater than approximately 50% in the headspace, consistent with results obtained with *M. thermoautotrophicum* (Daniels et al. 1977). The authors speculate that CO is metabolized according to Eqs. 2a and b, and the inhibition results from inhibition of hydrogenase catalyzing the oxidation of H_2 (O'Brien et al. 1984). Although the pathway for reduction of CO_2 to CH_4 with H_2 was not investigated, it is presumed to follow the well-studied pathway determined for freshwater methanogens, as shown in Fig. 3 for *Methanosarcina* species, with one possible exception. With H_2 as the electron donor, the EchA-F (*Escherichia coli*-type hydrogenase) complex oxidizes H_2 and reduces ferredoxin in reaction 1 (Fig. 3) that supplies electrons for reaction 2. The EchA-F complex is necessary, since the reduction of CO_2 with H_2 (reactions 1 plus 2) is thermodynamically unfavorable under standard conditions of one atmosphere H_2 ($\Delta G^{\circ\prime}$=+16 kJ/mole) and considerably more so with the much lower concentrations of H_2 in the environment. This thermodynamic barrier is overcome by reverse electron transport to ferredoxin driven by the electrochemical proton gradient and catalyzed by the EchA-F hydrogenase complex. However, when cultured with CO, it is presumed that ferredoxin is the electron acceptor of the CO dehydrogenase and the oxidation of CO coupled to reduction of CO_2 (reaction 2) becomes thermodynamically favorable ($\Delta G^{\circ\prime}$ =−4 kJ/mole) (Ferry and House 2006) obviating the requirement for EchA-F hydrogenase (Stojanowic and Hedderich 2004).

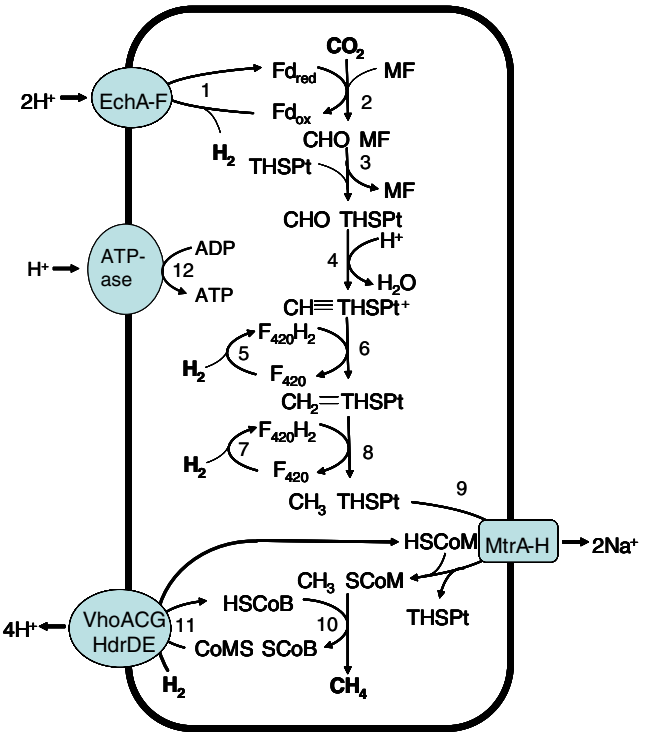

Fig. 3 The CO_2 reduction pathway of freshwater *Methanosarcina* species utilizing H_2. *Fd* Ferredoxin, *THSPt* tetrahydrosarcinapterin, *HS-CoB* coenzyme B, *HS-CoM* coenzyme M, *MF* methanofuran

Marine environments

Recent investigations of *Methanosarcina acetivorans* demonstrate that this marine isolate displays robust growth with CO as the sole carbon and energy source (Lessner et al. 2006; Rother et al. 2007). The generation time (~20 h) is less than that reported for other methanogens, and growth occurs with CO concentrations greater than 1 atmosphere. Although a few methanogens have been shown to utilize CO for growth, the experiments have routinely utilized concentrations above 50% in the atmosphere which is several-fold greater than is likely encountered in the environment. The lowest concentrations that support growth have not been reported for any methanogen, nor has a measurement of CO concentrations in the native habitats. Thus, a role for CO as the sole carbon and energy source in the native habitats of methanogens is still in question. Nonetheless, *M. acetivorans* was isolated from sediments rich in decaying kelp appended with flotation bladders containing up to 10% CO as a potential source of CO for growth of this marine isolate (Abbott and Hollenberg 1976; Sowers et al. 1984).

A biochemical and quantitative proteomic analysis of methanol- and acetate-grown versus CO-grown *M. acetivorans* (Lessner et al. 2006) has provided, for the first time, evidence of a pathway for CO-dependent methanogenesis supporting growth (Fig. 4) that has been confirmed in part by a qualitative proteomic analysis (Rother et al. 2007). In the pathway, 3 CO are oxidized to 3 CO_2 that provides electrons for the subsequent reduction of 1 CO_2 to form a methyl group attached to the cofactor tetrahydrosarcinapterin (THSPt) (reactions 1–6) similar to the pathway of freshwater methanogens (Fig. 3) except the electron donor. The methyl group of methyl-THSPt is transferred to coenzyme M (HS-CoM), although the route may be different from freshwater strains. The methyltransferase that transfers the methyl group from methyl-THSPt to HS-CoM (MtrA-H) in

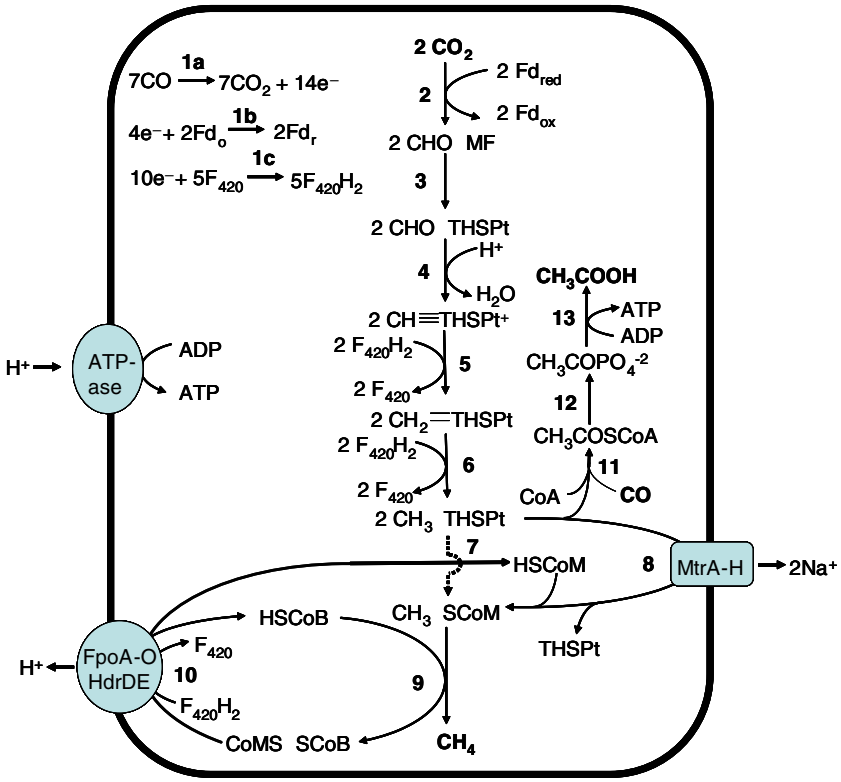

Fig. 4 Pathway for conversion of CO to acetate and methane by the marine isolate *Methanosarcina acetivorans*. *Fd* Ferredoxin, *THSPt* tetrahydrosarcinapterin, *HS-CoB* coenzyme B, *HS-CoM* coenzyme M, *MF* methanofuran

all known methanogenic pathways is down-regulated in CO-grown *M. acetivorans* versus methanol- and acetate-grown cells (Lessner et al. 2006) suggesting a reduced involvement of MtrA-H in the pathway (reaction 8, Fig. 4). The MtrA-H complex is membrane-bound and couples the methyl transfer reaction to translocation of sodium from the cytoplasm across the membrane to the periplasm, forming a gradient (Gottschalk and Thauer 2001). Indeed, the sodium requirement for methanogenesis is thought to originate from this enzyme, and the sodium gradient is postulated to drive energy-requiring reactions (Gottschalk and Thauer 2001). Three homologs annotated as putative corrinoid-containing proteins are up-regulated in CO-grown versus methanol- and acetate-grown *M. acetivorans* consistent with a role during growth with CO (Rother et al. 2007). Corrinoid cofactors bind the methyl group in a variety of methyltransferases from methanogens including the MtrA-H complex (Gottschalk and Thauer 2001); thus, it has been postulated (Lessner et al. 2006) that the up-regulated corrinoid proteins participate in transfer of the methyl group from methyl-THSPt to HS-CoM via a soluble sodium-independent pathway (reaction 7, Fig. 4) distinct from MtrA-H. In fact, one of the homologs overproduced in *Escherichia coli* (MA4384) catalyzes transfer of the methyl group from methyl-THSPt to HS-CoM (unpublished) supporting the proposal. The proposal does not rule out that both pathways may function simultaneously. Indeed, it is conceivable that both sodium-dependent MtrA-H and the postulated soluble sodium-independent pathway are required to accommodate variations in the energy available to pump sodium. Thus, at low environmental CO concentrations, the available energy is low and the transfer from methyl-THSPt to HS-CoM would necessarily shift more towards the sodium-independent pathway. With increased CO levels and greater available energy, methyl transfer would shift to the sodium-pumping MtrA-H complex to optimize the thermodynamic efficiency. Under laboratory conditions where cells are routinely cultured with greater than 0.5 atmosphere of CO, the postulated soluble sodium-independent route may be dispensable. The mechanism for providing electrons for reductive demethylation of CH_3-S-CoM to CH_4 is a prominent characteristic of the proposed pathway for conversion of CO to CH_4 in *M. acetivorans* that further distinguishes it from the well-characterized CO_2 reduction pathway of freshwater methanogens. In freshwater species of *Methanosarcina*, HS-CoB donates electrons for the reductive demethylation of CH_3-S-CoM to CH_4 catalyzed by methylreductase (reaction 10, Fig. 3). The heterodisulfide CoM-S-S-CoB is also a product of reaction 10 that is reduced to the active sulfhydryl forms of the cofactors catalyzed by the VhoACG/HdrDE complex (reaction 11, Fig. 3). H_2 is oxidized by the VhoACG hydrogenase with transfer of electrons to the heterodisulfide reductase HdrDE that is coupled to formation of an electrochemical potential that drives ATP synthesis (reaction 12, Fig. 3). On the other hand, the proteomic and biochemical evidence indicates that HdrDE and the FpoA-O complex ($F_{420}H_2$ dehydrogenase)

participate in the transfer of electrons to the heterodisulfide in *M. acetivorans* (reaction 10, Fig. 4). HdrDE and the FpoA-O complex also function in the pathway of methanol conversion to methane in *Methanosarcina* species (Ferry and Kastead 2007), including *M. acetivorans* (Li et al. 2007), wherein methanophenazine mediates electron transfer between FpoA-O and HdrDE. Methanophenazine is a quinone-like compound that translocates protons from the cytoplasm to the periplasm forming an electrochemical potential that drives ATP synthesis. Additionally, FpoA-O pumps protons contributing to the electrochemical potential in methanol-grown *Methanosarcina* species. Thus, it is postulated (Lessner et al. 2006) that the FpoA-O/HdrDE complex contributes to the electrochemical potential that drives ATP synthesis in CO-grown *M. acetivorans* (Fig. 4). The mechanism for the formation of formate is not known.

The products acetate and formate are yet another feature of the pathway in *M. acetivorans* that contrasts with *M. barkeri* (Rother and Metcalf 2004; Lessner et al. 2006; Oelgeschlager and Rother 2009). Quantitative proteomic analyses (Lessner et al. 2006) suggest a pathway in which CO dehydrogenase/acetyl-CoA synthase (CdhA-E) catalyzes synthesis of acetyl-CoA from the methyl group of methyl-THSPt, CO, and CoA-SH (reaction 11, Fig. 5). The acetyl-CoA is further converted to acetate catalyzed by phosphotransacetylase and acetate kinase (reactions 12 and 13) with the synthesis of ATP. Thus, it appears that ATP is synthesized via both substrate level and chemiosmotic mechanisms. The CdhA-E complexes from freshwater *Methanosarcina* species have CO dehydrogenase activity with ferredoxin as the electron acceptor (Terlesky and Ferry 1988b), as does the *M. acetivorans* enzyme (unpublished results) consistent with the CdhA-E also functioning in steps 1a and 1b (Fig. 4). Quantitative RTPCR analysis indicates modest up-regulation of two genes annotated as encoding CooS in CO-grown versus acetate-grown cells (Lessner et al. 2006). CooS is a CO dehydrogenase first described in *Rhodospirillum rubrum* (32) that also reduces ferredoxin; thus, the putative CooS in *M. acetivorans* is also a candidate for catalyzing reactions 1a and 1b. Phenotypic analyses of mutant strains of *M. acetivorans* deleted of the *cooS* genes suggest they contribute to but are not required for CO-dependent growth (Rother et al. 2007). There are no known enzymes in *M. acetivorans* responsible for reactions leading from CO to coenzyme F_{420}.

Notably, H_2 is not an intermediate in the pathway for CO conversion to CH_4 in *M. acetivorans* (Lessner et al. 2006) in contrast to freshwater *M. barkeri* (Fig. 3). Indeed,

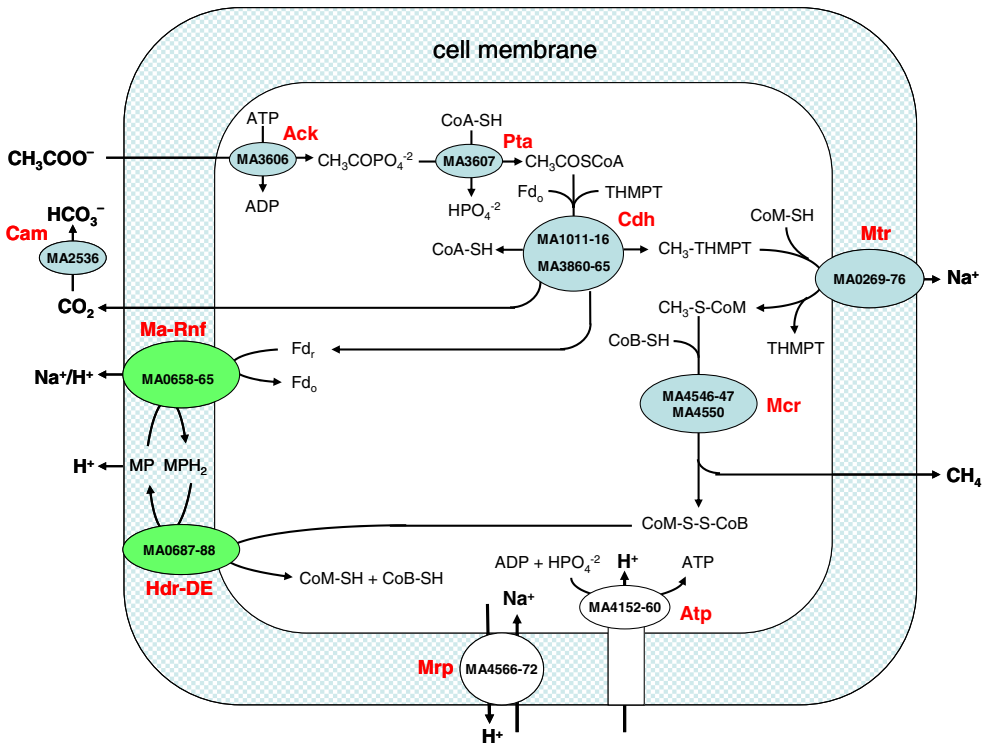

Fig. 5 Pathway for the conversion of acetate to methane by *Methanosarcina acetivorans*. *Ack* Acetate kinase, *Pta* phosphotransacetylase, *CoA-SH* coenzyme A, *THMPT* tetrahydromethanopterin, Fd_r reduced ferredoxin, Fd_o oxidized ferredoxin, *Cdh* CO dehydrogenase/acetyl-CoA synthase, *CoM-SH* coenzyme M, *Mtr* methyl-THMPT: CoM-SH methyltransferase, *CoB-SH* coenzyme B, *Cam* carbonic anhydrase, *Ma-Rnf M. acetivorans* Rnf, *MP* methanophenazine, *Hdr-DE* heterodisulfide reductase, *Mrp* multiple resistance/pH regulation Na^+/H^+ antiporter, *Atp* H^+-transporting ATP synthase. Carbon transfer reactions are catalyzed by the enzymes shown in *blue*. Electron transfer reactions are catalyzed by enzymes shown in *green*. By permission (Li et al. 2006)

M. acetivorans does not encode a functional EchA-F hydrogenase (Galagan et al. 2002). Thus, the observation that *M. acetivorans* is more tolerant of high concentrations of CO compared with *M. barkeri* is consistent with the postulated CO inhibition of hydrogenase in *M. barkeri* (O'Brien et al. 1984). The question then arises why *M. acetivorans* evolved a pathway for utilization of CO independent of H_2. One possibility is that, in marine environments, sulfate-reducing microbes outcompete methanogens for H_2 (Zinder 1993), presenting the possibility that if H_2 were an intermediate it could be lost to sulfate reducers. Another possibility is that *Methanosarcina* species are at a disadvantage utilizing H_2 in the marine environment where the concentrations are kept low by sulfate-reducers and, therefore, have not evolved hydrogenases. Indeed, it is noted that *Methanosarcina* species have considerably higher threshold concentrations for H_2 than do obligate CO_2-reducing species (Thauer et al. 2008).

The formation of methanethiol and dimethylsulfide during growth of *M. acetivorans* on CO was recently reported (Moran et al. 2008). The authors speculate that methyl-groups generated in the CO-dependent reduction of CO_2 are transferred to sulfide present in the growth medium and the process could be coupled to energy conservation. However, the rate of dimethylsulfide formation from CO is only 1–2% of that of CH_4 formation suggesting the process is not of major consequence in the energy yielding metabolism during growth on CO (Oelgeschlager and Rother 2009).

CO dehydrogenase/Acetyl-COA synthase

Conversion of acetate to CH_4

The five-subunit CdhA-E complex is central to pathways for conversion of acetate to methane (Kohler and Zehnder 1984; Krzycki and Zeikus 1984; Nelson and Ferry 1984; Krzycki et al. 1985; Bott et al. 1986; Terlesky et al. 1986; Bott and Thauer 1987; Zinder and Anguish 1992; Gokhale et al. 1993; Kemner and Zeikus 1994) as illustrated in Fig. 5 for *M. acetivorans* (Li et al. 2006). The complex functions in the pathway to cleave the C-C and C-S bonds of acetyl-CoA yielding a carbonyl group that is oxidized to CO_2 and a methyl group that is transferred to THSPt (Terlesky et al. 1987; Fischer and Thauer 1989; Abbanat and Ferry 1990; Raybuck et al. 1991; Grahame 1991, 1993; Grahame and Demoll 1995; Grahame et al. 1996; Bhaskar et al. 1998). In subsequent steps of the pathway, electrons derived from the oxidation of the carbonyl group are transferred to the methyl group producing CH_4. The complex also catalyzes the synthesis of acetyl-CoA from a methyl donor, CO and CoA-SH (Abbanat and Ferry 1990; Raybuck et al. 1991). Although a two-subunit CO dehydrogenase has been purified and characterized from an acetate-utilizing species from the genus *Methanosaeta* (formerly *Methanothrix*) (Boone and Kamagata 1998) that catalyzes the exchange of CO with the carbonyl group of acetyl-CoA (Jetten et al. 1989, 1991a, b; Eggen et al. 1991), the majority of mechanistic investigations have been with the five-subunit CdhA-E complexes from the acetate-utilizing species *Methanosarcina thermophila* and *M. barkeri* described here.

The subunits of the CdhABCDE complex from *Methanosarcina* species are correspondingly designated $\alpha\varepsilon\beta\gamma\delta$ and encoded in operons arranged in the order *cdhABCDE* (Maupin-Furlow and Ferry 1996a, b; Grahame et al. 2005). Use of a plasmid-mediated *lacZ* fusion reporter system has revealed that the operon encoding the complex of *M. thermophila* (Grahame et al. 2005) is 54-fold down-regulated in *M. acetivorans* grown on methanol compared to acetate, consistent with a role for the complex in the pathway for utilization of acetate (Apolinario et al. 2005). The results confirm an earlier report on the regulation of *cdhA* in *M. thermophila* examined by northern blotting (Sowers et al. 1993). Unlike the genome of *M. thermophila* that harbors only one operon encoding CdhA-E (Grahame et al. 2005), the genomes of *M. acetivorans* (Galagan et al. 2002) and *Methanosarcina mazei* (Deppenmeier et al. 2002) contain duplicate *cdh* operons with greater than 95% identity, raising the question as to whether both are transcribed during growth on acetate. A proteomic analysis of *M. acetivorans* indicates that one complex is expressed at least 16-fold over the other (Li et al. 2006, 2007), consistent with the predominance of a single Cdh complex purified from acetate-grown *M. mazei* strain Go1 (formerly *Methanosarcina frisia*) (Maestrojuan et al. 1992), although the organism encodes duplicate *cdh* operons with high identity (Eggen et al. 1996). However, in apparent contrast to this result, global transcriptional profiling of *M. mazei* Go1 suggests both *cdh* operons are transcribed at approximately equal levels (Hovey et al. 2005).

The complex from *Methanosarcina* species is resolvable into three components (Abbanat and Ferry 1991; Grahame and Demoll 1996; Kocsis et al. 1999). The CdhAE component contains the α and ε subunits, the CdhDE component contains the γ and δ subunits, and the CdhC component contains the β subunit. The CdhAE component has CO dehydrogenase activity with ferredoxin as the electron acceptor (Terlesky and Ferry 1988a, b; Fischer and Thauer 1990). The crystal structure (Gong et al. 2008) of the *M. barkeri* CdhAE (Fig. 6) shows a $\alpha_2\varepsilon_2$ configuration with the α subunit harboring a Ni-Fe-S C cluster and four Fe_4S_4 clusters (B, D, E, and F in Fig. 6) consistent with earlier EPR spectroscopic investigations (Krzycki et al. 1989). The C, B, and D clusters are posi-

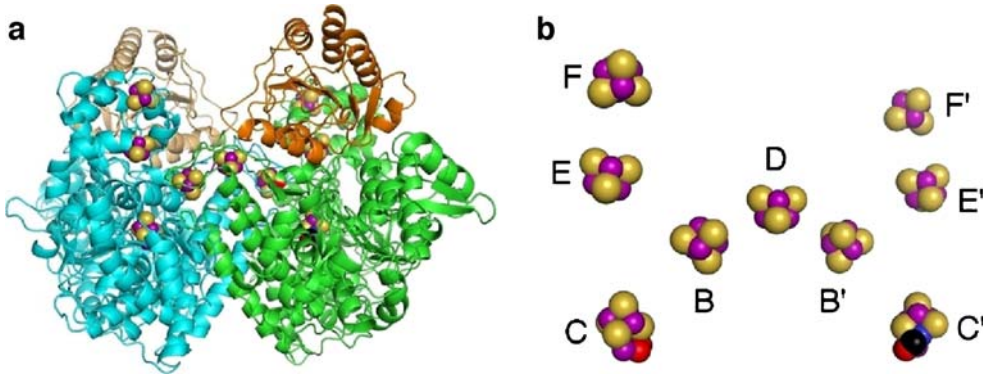

Fig. 6 The *Methanosarcina barkeri* $\alpha_2\varepsilon_2$ CdhAE component.**a** Side view shown as *ribbons* with the α-subunits colored in *cyan* and *green* and the ε-subunits in *tan* and *orange*. Metal cluster atoms are shown as *spheres*, with iron atoms in *purple*, nickel atoms in *blue*, and the remaining atoms in *CPK*. **b** Side view of the metal clusters. By permission (Gong et al. 2008)

tioned similarly in the crystal structure of the homolog from *Moorella thermoacetica* (Ragsdale 2007), an acetate-producing species of the *Bacteria* domain. However, the E and F clusters are unique to CdhAE and proposed to function in electron transport from the active site C cluster to ferredoxin. The C cluster (Fig. 7) resembles the *M. thermoacetica* structure comprised of a pseudocubane $NiFe_3S_4$ cluster bridged to an exogeneous iron atom. Additional electron density provides support for coupling between CO bound to the nickel and H_2O/OH^- bound to the exogenous iron in the critical C = O bond-forming step leading to CO_2 (Fig. 7). The structure also identifies a gas channel extending from the C cluster to the surface of the protein in a direction presumed to interact with the CdhC component. Structural and sequence alignments indicate that the ε-subunit is a member of the DHS-like NAD/FAD-binding domain clan. Indeed, the structure reveals a cavity able to accommodate an FAD or NAD cofactor located between the interface of the α- and ε-subunits and near the D Fe_4-S_4 cluster suggesting a potential role in electron transfer. The authors of the structure note that FAD is an electron acceptor for the $\alpha_2\varepsilon_2$ component (Grahame and Stadtman 1987) suggesting a potential role for the ε-subunit in FAD-mediated CO oxidation during growth on CO.

The CdhDE component transfers the methyl group of acetyl-CoA to THSPt and contains an iron-sulfur center and corrinoid cofactors that transfer the methyl group during catalysis (Grahame 1991, 1993; Jablonski et al. 1993; Maupin-Furlow and Ferry 1996a). Analyses of the CdhD and CdhE subunits overproduced independently in *E. coli* indicates that the iron-sulfur center is located in the CdhE subunit and that both subunits bind a corrinoid cofactor. Which of the two subunits interact with THSPt has not been determined. A structure has not been reported for either subunit; however, EPR analyses of the intact CdhDE component (Jablonski et al. 1993) indicate that the corrinoids are maintained in the base-off state with a E'_o of -486 mV for the $Co^{2+/1+}$ couple that facilitates reduction of Co^{2+} by approximately 12 kcal/mol relative to base-on cobamides. Reduction to the Co^{1+} redox state is a requirement for methylation of corrinoid cofactors. The EPR analysis also identified a $[4Fe\text{-}4S]^{2+/1+}$ cluster with an E'_o of -502 mV,

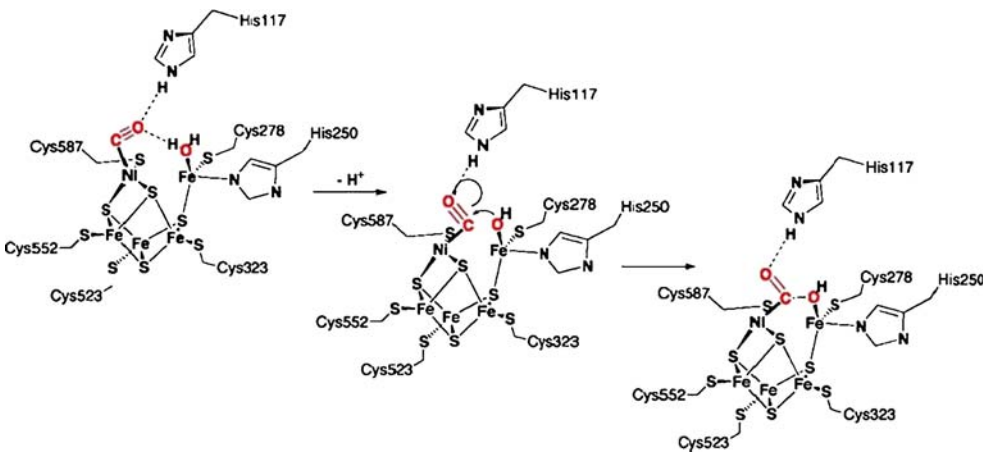

Fig. 7 Proposed coupling of the CO and H_2O species in the C cluster of the *Methanosarcina barkeri* CdhAE component

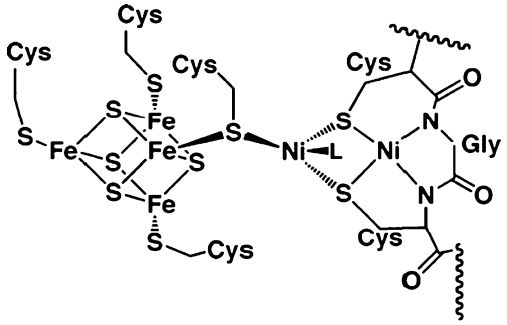

Fig. 8 Structure of the A cluster from *Moorella thermoacetica*. By permission (Ragsdale 2007)

approximately isopotential with the $Co^{2+/1+}$ couple suggesting the cluster is likely involved in reducing Co^{2+}.

The CdhC component contains an A cluster that is the proposed site of acetyl-CoA cleavage or synthesis (Grahame and Demoll 1996; Murakami and Ragsdale 2000; Gencic and Grahame 2003; Funk et al. 2004). Although a structure is not available, a variety of spectroscopic studies suggest the A cluster is comprised of an Fe_4S_4 center bridged to a binuclear Ni-Ni site (Gu et al. 2003; Funk et al. 2004) similar in structure (Fig. 8) to that proposed for the homolog from *M. thermoacetica* (Ragsdale 2007). In addition to CO oxidation, the CdhAE component is required for acetyl-CoA cleavage or synthesis (Murakami and Ragsdale 2000). The authors propose a mechanism for acetyl-CoA synthesis or cleavage involving an intramolecular electron transfer reaction between the C cluster in the CdhAE component and cluster A in the CdhC component for each catalytic cycle thereby maintaining Ni in cluster A in the catalytically active Ni(I) redox state. The redox dependence of acetyl-CoA synthesis by the CdhC component shows one-electron Nernst behavior and that two electrons are required for reductive activation of the active site Ni in the process of forming the enzyme-acetyl intermediate (Gencic and Grahame 2008).

Autotrophy

Many methanogens grow autotrophically with CO_2 as sole source of carbon (Ferry and Kastead 2007). For example, *M. thermoautotrophicum* is able to grow with H_2 and CO_2 in a simple mineral salts medium (Zeikus and Wolfe 1972; Schonheit et al. 1980) synthesizing acetyl-CoA (Stupperich and Fuchs 1983; Stupperich et al. 1983; Ruhlemann et al. 1985), the starting point for synthesis of all cellular components (Fuchs and Stupperich 1980). Acetyl-CoA is synthesized via methyl-tetrahydromethanopterin (methyl-THMPt) providing the methyl group and CO the carboxyl group (Lange and Fuchs 1985, 1987). The CO is generated from CO_2 and H_2 in an energy-dependent reaction (Conrad and Thauer 1983; Eikmanns et al. 1985) and methyl-THMPt *via* steps in the pathway for CO_2 reduction to methane (Lange and Fuchs 1987) similar to that shown in Fig. 3 (reactions 2–8). The THMPt in *M. thermoautotrophicum* is an analog of THSPt found in *Methanosarcina* species. Autotrophic methanogens such as *M. thermoautotrophicum* have CO dehydrogenase activity whereas heterotrophic methanogens lack CO dehydrogenase and require acetate as the carbon source that is converted to acetyl-CoA (Bott et al. 1985). Indeed, the genome of *M. thermoautotrophicum* contains a gene cluster encoding subunits with high identity to subunits of the CdhA-E

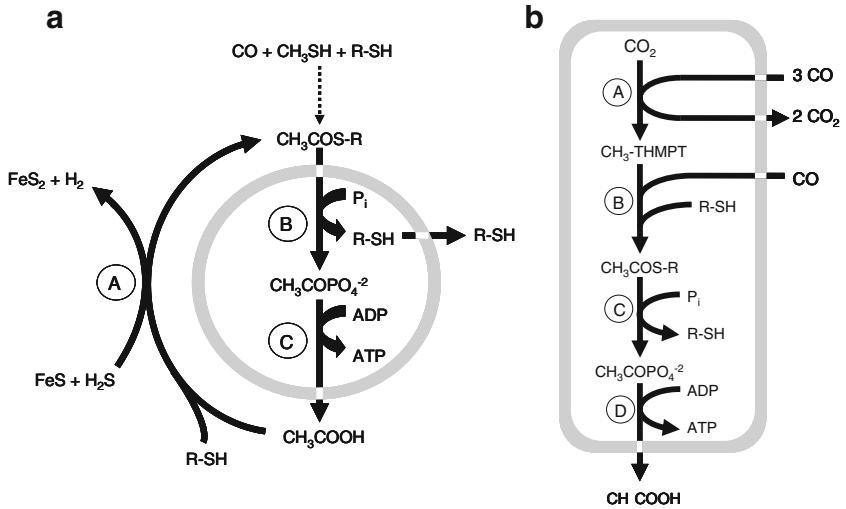

Fig. 9 The proposed cyclic energy-conserving pathway that functioned in the primitive (**a**) and chemoautotrophic cell independent of surface-catalyzed reactions (**b**) (Ferry and House 2006). The *stippled areas* represent the lipid membranes. *Pi* represents inorganic phosphate. a *Solid lines* indicate the cyclic pathway (steps *A–C*). The *broken arrow* indicates the priming reaction that is not part of the cyclic pathway. **b** *THMPT* Tetrahydromethanopterin. By permission (Ferry and House 2006)

complex of acetate-utilizing *Methanosarcina* species (The Comprehensive Microbial Resource. J. Craig Venter Institute, http://cmr.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi). Furthermore, a CO dehydrogenase was purified from the autotrophic methanogen *Methanococcus vannielii* that contains nickel and is composed of subunits with molecular weights similar to the CdhAE component (DeMoll et al. 1987). These results are consistent with synthesis of acetyl-CoA in autotrophic methanogens catalyzed by a CO dehydrogenase with properties similar to the CdhA-E complex of acetate-utilizing species.

Evolution

It is proposed that Earth's atmosphere at the time of the origin of life contained significant amounts of CO (Holland 1984; Kasting 1990; Kharecha et al. 2005) and that the CdhA-E complex of methanogens and homologs in the *Bacteria* domain evolved early during the origin and evolution of primitive life forms (Lindahl and Chang 2001). In the chemoautotrophic origin of life (Russell et al. 1988; Wachtershauser 1988; Russell and Hall 1997), the primary pre-biotic initiation reaction for carbon fixation was the surface-catalyzed synthesis of an acetate thioester from CO and H_2S driven by a geochemical energy source. A prominent feature of the chemoautotrophic theory is the evolution of biological CO_2 fixation pathways of which one is a primitive form of the extant Wood–Ljungdahl pathway (Wachtershauser 1997; Pereto et al. 1999; Martin and Russell 2003; Russell and Martin 2004) in which $2CO_2$ molecules are reduced to acetyl-CoA implying early evolution of primitive forms of enzymes with catalytic capabilities of the CdhA-E complex of methanogens and homologs in the *Bacteria* domain. A role for a primitive form of CdhC has been proposed in a modification of the chemoautotrophic theory (Ferry and House 2006). In the modified theory, the primitive CdhC catalyzes C-S bond formation (reaction A in Fig. 9a) regenerating the CH_3COSR thioester from acetate and HS-R that is the energy source in the first energy-yielding metabolic cycle. Reaction A in Fig. 9a is driven by the conversion of FeS and H_2S to pyrite and H_2 a previously proposed energy source in the chemoautotrophic theory (Wachtershauser 1988). The theory also proposes roles for primitive forms of phosphotransacetylase and acetate kinase (reactions B and C, Fig. 9a). In contrast to biosynthetic pathways, the cycle is proposed to have been the major force that powered and directed the early evolution of life that included acetogens and methanogens (Ferry and House 2006). The next proposed step in evolution of extant CdhA-E and its homologs in the *Bacteria* domain (Ferry and House 2006) is evolution of CO dehydrogenase activity (reaction A, Fig. 9b) and C-C bond formation (reaction B, Fig. 9b) that allowed the synthesis of thioesters from CO and a methyl group derived from co-evolution of enzymes leading to the reduction of CO_2 to the methyl level that is the extant Wood-Ljungdahl pathway (Fig. 9b). Thus, it is proposed that the Wood-Ljungdahl pathway first evolved as an energy-yielding pathway that was adapted for biosynthesis of cell carbon, and evolution of the first autotroph, once the pre-biotic organic soup was exhausted for incorporation into cell material (Ferry and House 2006). It is further proposed that the pathway shown in Fig. 9b was the foundation for evolution of methanogenic pathways.

Conclusions

Methanogens play an essential role in the global carbon cycle by serving as terminal organisms in anaerobic microbial food chains that convert complex organic matter to CH_4 and CO_2. They utilize simple molecules for growth that include acetate and several one-carbon compounds. Although first reported in 1931, comparatively little is known today of the ecology, physiology, and biochemistry of CO utilization by methanogens. Only a few species are reported to metabolize CO, and it is not yet known unequivocally if CO is a viable energy source for methanogens in diverse environments. However, recent investigations of *M. acetivorans* suggest this methanogen utilizes CO as a growth substrate in nature converting CO, abundant in the native habitat, to CH_4 and acetate via an unusual pathway. CO is also an important intermediate during growth of methanogens on acetate and assimilation of CO_2 for cell carbon. Clearly, more research is warranted to determine the extent of CO utilization by methanogens in native environments.

Acknowledgements Research in the laboratory of J.G.F has been supported by the NIH, DOE, NSF, and NASA.

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