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Single-Carbon Chemistry of Acetogenic and Methanogenic Bacteria

J. G. Zeikus, R. Kerby, J. A. Krzycki

Summary. Methanogenic and acetogenic bacteria metabolize carbon monoxide, methanol, formate, hydrogen and carbon dioxide gases and, in the case of certain methanogens, acetate, by single-carbon (C_1) biochemical mechanisms. Many of these reactions occur while the C_1 compounds are linked to pteridine derivatives and tetrapyrrole coenzymes, including corrinoids, which are used to generate, reduce, or carbonylate methyl groups. Several metalloenzymes, including a nickel-containing carbon monoxide dehydrogenase, are used in both catabolic and anabolic oxidoreductase reactions. We propose biochemical models for coupling carbon and electron flow to energy conservation during growth on C_1 compounds based on the carbon flow pathways inherent to acetogenic and methanogenic metabolism. Biological catalysts are therefore available which are comparable to those currently in use in the Monsanto process. The potentials and limitations of developing biotechnology based on these organisms or their enzymes and coenzymes are discussed.

There has been considerable chemical research on the technological application of single-carbon (C_1) transformations. Synthesis gas (syngas), a mixture of hydrogen and carbon monoxide has long been used as a feedstock for production of industrial chemicals. Although syngas has been derived from methane it is increasingly manufactured from coal and may be derived from nonfossilized forms of biomass. The role of syngas and C_1 chemistry in the production of polymeric chemicals and fuels may increase as the supply of petroleum-derived chemicals becomes limiting (*1*).

The conversion of single-carbon compounds to higher molecular weight products depends on metal-containing catalysts (*2*). The conversion of syngas to methane and higher hydrocarbons (Fischer-Tropsch reaction) and of methane to ethanol occur in the presence of iron-, cobalt-, or nickel-containing catalysts. The conversion of methanol (itself derived from syngas by copper-zinc chromite–containing catalysts) and CO to acetic acid depends on homogeneous rhodium-containing catalysts (Monsanto process), although cobalt was used initially and efforts to produce suitable nickel-containing catalysts are under way.

There are similarities between many of these C_1 transformation reactions and metabolic reactions occurring in at least two groups of anaerobic bacteria (acetogens and methanogens) that consume various single-carbon substrates and acetate as energy sources. The acetogens produce acetic, butyric, or mixtures of both acids, while the methanogens produce methane. These bacteria are notable for (i) their complements of unusual enzymes and coenzymes in which nickel, cobalt, iron, tungsten, molybdenum, selenium, and zinc are present either singly or in various combinations; and (ii) their unusual energy-yielding mechanisms, which do not necessarily rely on the breakdown of carbon substrates. Thus, the usual connection between catabolism and degradation of carbon-carbon bonds is not found in the catabolic processes of acetogens and methanogens when they are grown on C_1 substrates.

Early studies on acetogens and methanogens have followed a fairly parallel course because they are found in similar environments and because they utilize some of the same substrates (*3*). Thus, research on cultures enriched for growth of methanogens (*3*) recorded methane and "vinegar acid" formation from H_2 and CO_2. Soon representative species were isolated including the acetogen *Clostridium aceticum*, which consumes saccharides and H_2 plus CO_2, and the methanol- and acetate-catabolizing methanogen, *Methanosarcina barkeri*.

We review the C_1 chemistry of anaerobes that can consume C_1 compounds as their sole electron donors and acceptors including both acetogens and methanogens; however, we exclude those species in which sulfate is used as an electron acceptor (*4*) and the acetogens that degrade purines and amino acids (*5*).

Microbial Acetogenic Transformations

Table 1 (top) shows the physiological properties of widely studied acetogenic bacteria. Many other species have also been described, including *Clostridium thermoautotrophicum* which can grow on methanol at temperatures higher than 60°C (*6*). Analysis of RNA sequences from diverse acetogens has not revealed a unifying correlation between this catabolic process and phylogeny (*7*). As in

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Table 1. Physiological properties of representative acetogenic and methanogenic bacteria. The energy source for cell growth is listed in parentheses (G, glucose; F, fructose; A, acetate). When H_2 is the energy source, CO_2 is the carbon source.

Species	Optimal growth (°C)	Wall type	DNA (% G+C)	Corrinoid content* (nmoles)	Catabolic enzyme activities† (μmole/min per mg protein)				References
					CODH	HDH	FDH	MR‡	
				Acetogens					
Clostridium thermoaceticum	55–60	Peptidoglycan A3 γ	54	0.44–0.63 (G)	13 (G)	4 (G)	2.3 (G)		*15, 17, 64, 65*
Acetobacterium woodii	30–35	Peptidoglycan B2 α	39	1.21–1.36 (F)	20 (F)	25 (H_2)	3–3.9 (F)		*8, 16, 66*
Butyribacterium methylotrophicum	37–40	Peptidoglycan B2 α	50	0.5 (G)	13 (CO)	0.9 (CO)	3.8 (CO)		*9, 65*
				11.2 (CH_3OH)	47 (CH_3OH)	2.4 (CH_3OH)	1.0 (CH_3OH)		
				Methanogens					
Methanosarcina barkeri strain MS	37–40	Heteropolysaccharide	44	3.2 (A)	1.0 (H_2)	6.0 (H_2)		0.0005 (H_2)	*27, 47, 52, 67, 68*
				8.2 (CH_3OH)	5.0 (A)				
Methanobacterium thermoautotrophicum strain ΔH	65–70	Pseudomurein	52	1.3 (H_2)	0.2 (H_2)	47 (H_2)		0.010 (H_2)	*23, 27, 68, 69*

*Data are expressed per milligram of cell protein. †CODH, CO dehydrogenase; HDH, hydrogenase; FDH, formate dehydrogenase; MR, methylreductase. With the exception of MR, enzyme activities were measured in the direction of substrate oxidation coupled with the reduction of the low potential electron acceptor, methyl viologen. Because enzymes from different organisms have different affinities for this electron acceptor, comparison between organisms is unreliable. All activities are measured in crude extracts at the organism's optimal growth temperature. ‡Units are μmole of CH_4 produced per minute per milligram of protein from methyl CoM in crude extracts in the presence of hydrogen gas.

other eubacteria, acetogens have typical ester-linked alkyl lipids and a peptidoglycan wall constructed from polymerized *N*-acetylmuramic acid and *N*-acetylglucosamine residues. The cross-linking bridge peptides (*8*), however, contain unusual sequences (Table 1).

Butyribacterium methylotrophicum is the most metabolically versatile acetogen described to date in that it uses a wide variety of C_1 substrates singly or in combination and, depending on the substrate composition, it can form acetate or butyrate as the sole end product, or mixtures of both acids (*9*). The metabolic processes for various single carbon substrate transformations of *B. methylotrophicum* with the energetics and growth parameters are shown in Table 2. For the organism to grow readily at high CO partial pressures (>1 atmosphere) it was necessary to select a CO-tolerant strain. The growth yield appears to be directly related to the free energy available from the various catabolic reactions and, when grown on a medium supplied with CH_3OH and CO_2, more than 20 percent of the CH_3OH is converted into cellular components. This represents a very high efficiency of cellular carbon synthesis for an anaerobic microorganism.

The most oxidized substrates are consumed by *B. methylotrophicum* either individually or concurrently, resulting predominantly in acetic acid synthesis (*9*). Formate and carbon monoxide (CO), although of equal oxidation state, are metabolized differently by *B. methylotrophicum* in that (i) more than twice as much CO as formate is consumed when both are present, and (ii) the consumption of formate gives rise to a large amount of H_2, which is consumed in the latter stages of fermentation, whereas H_2 is only detected as a trace product of CO consumption at the end of fermentation.

Substantial growth on methanol, a substrate more reduced per carbon atom than either acetate or butyrate, requires an oxidized co-substrate (such as CO_2, CO, formate, or acetate). Methanol and the co-substrate are simultaneously consumed. The transformation of methanol and CO during growth is a biochemical curiosity since, unlike typical fermentations, neither substrate needs to be oxidized or reduced when both are directly condensed to acetate. ^{13}C-nuclear magnetic resonance labeling studies show that labeled methanol is incorporated predominantly as the methyl moiety of acetate whereas CO is incorporated predominantly as the carboxyl moiety of acetate.

Research on growth and metabolism based on enzyme activities, as well as fermentation and substrate-product labeling patterns (Tables 1 and 2) have led to a common scheme for single-carbon transformations by acetogenic bacteria (Fig. 1). This model shows entry points for various substrates and three mechanisms of substrate-level adenosine triphosphate (ATP) generation: (i) the conversion of acetyl-coenzyme A (-CoA) to acetate and ATP via acetyl phosphate, (ii) conversion of butyryl-CoA to butyrate and ATP, and (iii) during methanol oxidation, the conversion of formyl-tetrahydrofolate (formyl-THF) to formate and THF by formyl-THF synthetase. These mechanisms of ATP generation may be involved in the synthesis of ATP by acetogens that metabolize purines (*5*). The initial reduced product of this catabolic process, acetyl-CoA, also serves as the precursor for cellular carbon (*10*) and thus efficiently links the catabolic and anabolic pathways. Tetrahydrofolate appears to play a key role in the transformation of C_1 units between the methyl and formyl oxidation levels in acetogens (Fig. 2). Acetogens have remarkably higher levels of these enzymes than other organisms (*11, 12*). Additional information is available concerning the biochemical details of anaerobic synthesis of acetic acid from C_1 compounds (*12–14*).

Hydrogen oxidation by hydrogenase (Fig. 1, reaction 1) is coupled with CO_2 reduction to acetate in acetogens. However, less is known about this enzyme from acetogens as compared with those from methanogens. Previous reports indicate that the hydrogenases of *Acetobacterium woodii* and *Clostridium thermoaceticum* are not nickel-containing enzymes (*15, 16*). Recent data support the presence of multiple hydrogenases in *C. thermoaceticum*, one of which is induced only in the presence of CO.

Formate dehydrogenase (FDH) functions at the oxidized end of the THF sequence (Fig. 1, reaction 3) by coupling the oxidation of formic acid to CO_2 with the reduction of an electron acceptor (*17*). The enzyme functions in the reverse direction under different reaction conditions (*18*). The oxygen-labile FDH from *C. thermoaceticum* has been purified (*17*) and the molar ratio of tungsten, selenium, iron, and sulfur present is approximately 2:2:36:50. The specific activity of the enzyme from different acetogens varies, depending both on the energy source (Table 1) and the concentrations of iron, selenium, tungsten, and molybdenum supplied in the growth medium. For example, the specific activity of FDH in extracts of fructose-grown *Clostridium formicoaceticum* can vary

by a factor of 250, depending on the metals present in the medium (*19*).

At the opposite end of the THF sequence lies a cobalt-containing corrinoid protein (labeled X in Fig. 2) required in the transfer of the methyl group from methyl-THF to the C-2 position of acetate (*20*). This protein is oxygen-labile and has only recently been purified. Although biochemical studies of the function of cobalt-corrinoids have been limited to *C. thermoaceticum*, all acetogens assayed had a higher content of corrinoids (see Table 1) than most other bacteria.

The mechanism of synthesis of the precursor to the acetate carboxyl group requires a carbon monoxide dehydrogenase (CODH) activity whose properties have been reviewed (*21*). Typically, CODH is assayed by coupling the oxidation of CO with the reduction of an artificial electron acceptor (Table 1). The CODH enzymes (*22*) from both *C. thermoaceticum* and *A. woodii* have been purified to >95 percent purity. The high molecular weight enzymes (440,000 and 460,000, respectively) contain nickel, zinc, iron, and acid-labile sulfur. Iron and sulfur exist as Fe_4S_4 clusters in these enzymes and are reduced and oxidized in the presence of CO and CO_2, respectively. On exposure to CO, both enzymes exhibit electron paramagnetic resonance (EPR) signals consistent with the formation of a nickel-carbon radical species.

The function of CODH in acetogens in vivo appears to be more extensive than the oxidation of CO to CO_2 (*9, 13, 23*) as indicated by (i) in vitro results indicating that CODH is one component necessary for the conversion of methyl-THF and either CO or the carboxyl group of pyruvate into acetyl-CoA; (ii) higher specific activities of the enzyme found when *B. methylotrophicum* was grown with methanol rather than CO as the energy substrate and the presence of significant amounts of the enzyme in all acetogens tested, regardless of growth substrate (Table 1); and, (iii) preferential in vivo incorporation of CO into the acetate carboxyl group by *A. woodii* and *B. methylotrophicum*. Furthermore, the effects of removal of nickel on the fermentation of fructose by *A. woodii* are consistent with a role for CODH in the synthesis of the acetate carboxyl group (*16*). Thus, it has been suggested that the role of CODH involves the synthesis of a carbonyl intermediate ([CO] in Fig. 1) from CO, CO_2, and the pyruvate carboxyl group (*21*). CODH and the cobalt-containing corrinoid protein may exist as a complex (*20*); however, the synthesis of the methyl-carbonyl bond of acetyl-CoA may be a corrinoid-dependent reaction independent of CODH (*24*).

Figure 2 is a model that can account

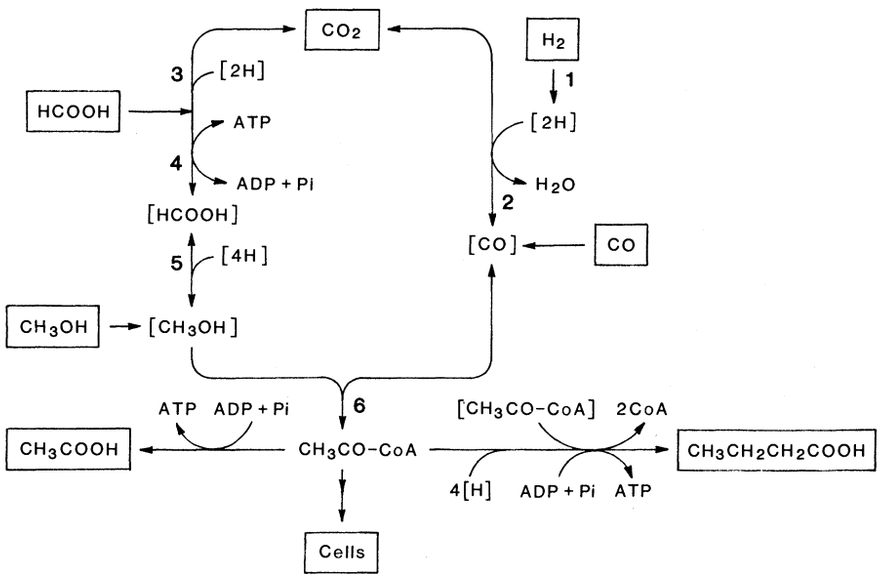


Fig. 1. Model of C_1 metabolism proposed for acetogenic bacteria that synthesize acetate or butyrate during growth on C_1 substrates or H_2 and CO_2. The symbols [CH_3OH] and [HCOOH] represent two oxidation states of C_1 units bound to tetrahydrofolate (THF) carriers while the chemical nature of [CO] remains undetermined. Numbers indicate the following enzymic activities: 1, hydrogenase; 2, CO dehydrogenase; 3, formate dehydrogenase; 4, formyl-THF synthetase; 5, other THF enzymes; and 6, one or more enzymes required for the synthesis of acetyl-CoA from [CO] and a methyl-corrinoid. The synthesis of acetyl CoA as a primary intermediary metabolite links both catabolic and anabolic processes to a common C_1-based chemistry.

Table 2. Single-carbon transformations associated with growth and energy conservation of acetogens and methanogens.

Balanced catabolic reactions*	$\Delta G^{\circ\prime}$ (kJ per mole substrate)†	Doubling time (hours)	Yield‡ (g cells per mole substrate)	References
Butyribacterium methylotrophicum				
$4HCOO^- + H_2O \rightarrow CH_3COO^- + 2HCO_3^- + OH^-$	−14.8	10	1.1	*9*
$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$	−28.0	10	1.7	
$4CO + 4H_2O \rightarrow CH_3COO^- + 2HCO_3^- + 3H^+$	−41.4	10	3.4	
$CH_3OH + CO \rightarrow CH_3COO^- + H^+$§	−48.5	11	4.6	
$10CH_3OH + 2CO_2 \rightarrow 3CH_3CH_2CH_2COO^- + 3H^+ + 8H_2O$‖	−54.8	8	7.6	
Methanosarcina barkeri strain MS¶				
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−34.7	12	1.6	*14, 52, 67, 70*
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	−77.7	10–24	3.9–5.4	
$CH_3COOH \rightarrow CH_4 + CO_2$	−27.6	60	3.9	
$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	−46.4	65	2.5	
$4CH_3NH_2 + 2H_2O \rightarrow 3CH_4 + CO_2 + 4NH_3$	−75.2	45	4.4	

*Theoretical equations are presented. In actual fermentations, a significant portion of the substrate carbon is incorporated into the cell mass, significantly altering the balanced fermentations reported here. †Based on 1 mole of the energy yielding substrate. For $CH_3OH + CO$ the amount of oxidized substrate for both the $\Delta G^{\circ\prime}$ and yield calculations was calculated as the sum of the CH_3OH and CO consumed. Thus, the $\Delta G^{\circ\prime}$ for the overall reaction is −96.7 kJ and the yield per mole of either substrate is 9.2 g cells. ‡Cell yield as measured in batch culture. §When formate acts as the cosubstrate for methanol consumption, the major product is butyrate. ‖Other acetogens only produce acetate, in which case the reaction stoichiometry becomes $4CH_3OH + 2CO_2 \rightarrow 3CH_3COO^- + 3H^+ + 2H_2O$. ¶In addition to the substrates listed, *M. barkeri* can use $(CH_3)_2NH$, $(CH_3)_3N$, and dimethylethylamine. Ethylamine is an end product of the catabolism of dimethylethylamine.

for energy conservation during growth of acetogens on carbon monoxide. Similar mechanisms could be devised to explain energy generation on formate or H_2-CO_2. Electron transport phosphorylation may be linked to the dehydrogenative (CODH) and hydrogenative (THF-linked reductions) reactions because substrate level phosphorylations would consume one ATP by formyl-THF synthetase (Fig. 2, reaction 3) for each ATP produced by acetate kinase (Fig. 2, reaction 9). Numerous soluble (ferredoxin, flavodoxin, and rubredoxin) and membrane bound (cytochrome b and menaquinone) electron carriers (*25*) have been detected in acetogens, but their function in coupling vectorial electron transfer to ATP synthesis has not been demonstrated.

Microbial Methanogenic Transformations

Methanogens display great diversity in morphology, ultrastructure, wall and membrane chemistry, and nucleic acid homology and more than ten different genera have been described (*14, 26–30*). Studies of RNA sequences from methanogens suggest that individual genera are phylogenetically separated but all genera have been assigned to the kingdom Archaebacteria and not to the kingdom Eubacteria (*31*). Methanogen membrane lipids are composed of ether-linked isoprenoid units (*32*) and their walls lack peptidoglycan but are composed of various polysaccharides, polypeptides, or a mixture of *N*-acetyltalosaminuronic acid, *N*-acetylglucosamine, and polypeptides (*33*).

Metabolic studies of the two best-characterized methanogenic species, *Methanosarcina barkeri* and *Methanobacterium thermoautotrophicum* (Table 1, bottom) and other methanogens, support the model for their single carbon biochemistry outlined in Fig. 3. Carbon flow from C_1 substrates to either a methyl or carbonyl intermediate or a C_2 product appears schematically similar to that shown in acetogens; however, the carbon carriers used are distinct from THF derivatives.

Barker first postulated that conversion of CO_2, methanol, or acetate to methane took place on carbon carriers (*26*). The initial steps of CO_2 reduction by H_2 oxidation are not well characterized, but CO_2 is thought to be activated by carbon dioxide reduction factor (CDR factor), an unusual coenzyme now identified as methanofuran (*34*). After reduction, the C_1 unit is transferred to tetrahydromethanopterin (THMP), also known as formaldehyde activation factor (*30, 35*). This pteridine is a yellow fluorescent coenzyme in methanogenesis (*36*) and has been characterized as methenyl THMP (*31*). Further reduction to the methyl level presumably takes place on THMP and the methyl group subsequently is transferred to mercaptoethanesulfonic acid (coenzyme M or CoM), a cofactor present in all methanogens (*37*). Methylmercaptoethanesulfonic acid (methyl CoM) and methenyl THMP are readily labeled when cells of *M. barkeri* are incubated with $^{14}CO_2$ or $^{14}CH_3OH$ (*36, 38*). Methyl CoM is reduced to methane by the enzyme methylreductase, which is present in *Methanosarcina* (Table 1, bottom) and can account for up to 12 percent of the soluble protein of *M. thermoautotrophicum* (*39*). Methylreductase contains a nickel tetrapyrrole, factor F_{430}, as its prosthetic group with 2 moles of the tetrapyrrole present per mole of enzyme (*40*). The manner in which factor F_{430} interacts with methyl CoM during methane production is controversial (*41*).

The reducing equivalents for the reduction of CO_2 to methane in *M. barkeri* and other methanogens are generated through the action of hydrogenase. Methanogens have at least two chromatographically distinct hydrogenases with different electron acceptor specificities (*42*). Molecular sizes of these hydrogenases may be $\leq 10^6$ daltons (*29*) and, like the hydrogenases of other organisms, they contain iron and sulfur. The nickel found in a hydrogenase isolated from *M. thermoautotrophicum* may act in electron transfer since a change in the redox state of the metal was noted after addition of substrate.

Formate, methanol, acetate, and carbon monoxide are transformed into methane by a series of reactions in which the initial steps differ from those occurring in the transformation of H_2 and CO_2. Formate is oxidized by FDH and the reducing equivalents thus generated are then used to reduce carbon dioxide (or perhaps formate directly) to methane. Two FDH's have been purified from *Methanococcus vannielii*, one of which contains iron, sulfur, and molybdenum, while the other contains, in addition, selenium and tungsten (*43*). Formate dehydrogenase from *Methanobacterium formicicum* has also been purified and contains iron, sulfur, and molybdenum (*44*). Both formate dehydrogenase and hydrogenase are linked to factor F_{420}, a low-potential electron carrier present in methanogenic bacteria (*43, 45*).

The metabolism of methanol (Fig. 3) is initiated by two methyl transferases (*30, 46*), which either act on or contain 5-hydroxybenzimidazoylcobamide (a derivative of vitamin B_{12}, a cobalt tetrapyrrole, or corrinoid). Methyltransferase I (MTI) was first described in *M. barkeri* by Blaylock, and has been characterized (*46*); it transforms methanol into an enzyme-bound methyl-corrinoid complex which is then transferred by methyltransferase II to CoM.

Methanogens contain large amounts of corrinoids (*14, 47*) which may function as coenzymes for both cellular carbon synthesis from C_1 substrates, and for methanogenesis from methanol and acetate (*14, 29, 30*). Iodopropane, a vitamin B_{12} antagonist, inhibits growth of *M. barkeri* and other methanogens on H_2 and CO_2, but this inhibition is relieved by addition of acetate to the growth medium. Iodopropane inhibits methanogenesis from acetate and methanol much more than from H_2 and CO_2 (*48*). The role of corrinoids in methanogenesis from H_2 and CO_2 remains unclear, but addition of vitamin B_{12} results in a 12-fold stimulation of CH_4 production by the purified methylreductase system of *Methanobacterium* (*49*).

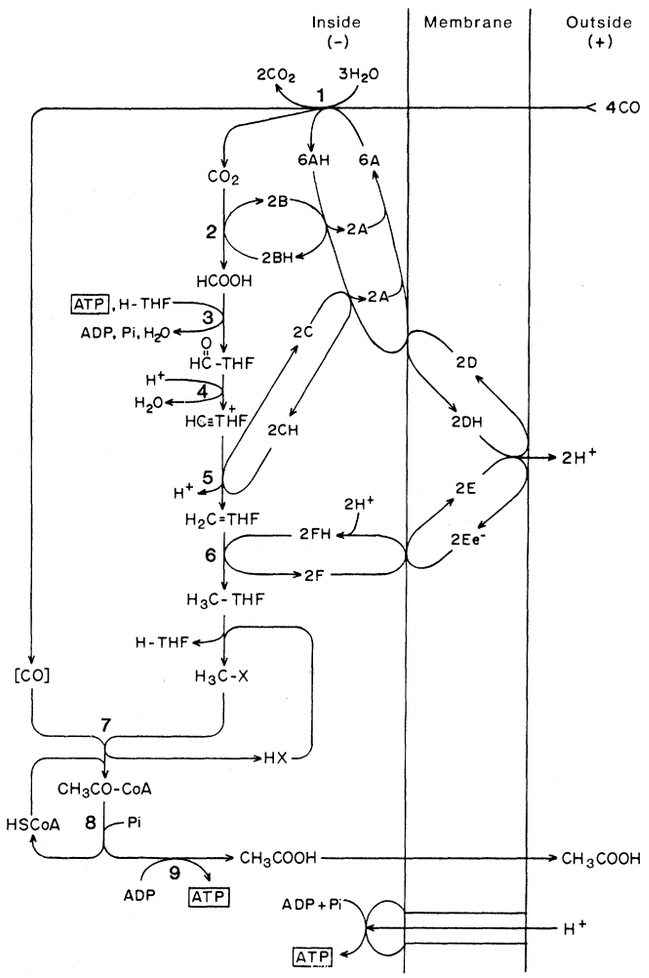
Methanogens including both *M. barkeri* and *M. thermoautotrophicum* synthesize cell precursors by C_1 transformation reactions that schematically resemble those of acetogens in the utilization of catabolic reactions and a methyl-carbonyl condensation reaction for C_2 synthesis. CO dehydrogenase is thought to function in anabolism through the synthesis of a carbonyl group which is the direct precursor of the carbonyl of acetyl CoA or the carboxyl of acetate (*14, 21, 50, 51*). Cell extracts of *M. barkeri* readily produce acetate from methyl-vitamin B_{12} and CO (*50*). Cyanide, an inhibitor of CO dehydrogenase, stops acetyl CoA synthesis from CO_2 and a methane precursor in cell extracts of *M. thermoautotrophicum*, but this inhibition is relieved by CO addition (*51*).

Methanosarcina barkeri also grows by the degradation of acetate to methane and carbon dioxide. The chemistry of this process has been suggested to proceed by a reversal of the reactions for acetate synthesis from C_1 substrates (*14, 21, 52, 53*). Acetate can be activated in cell-free extracts by acetate kinase and phosphotransacetylase (*51*), although a catabolic function for these enzymes has not been demonstrated. In Fig. 4 we present a model that accounts for energy conservation and the function of CO dehydrogenase during growth of methanogens on acetate. The specific activity of CO dehydrogenase increases fivefold when *M. barkeri* is grown on acetate

rather than H_2 and CO_2, although the doubling time on acetate is much longer (*52*); this enzyme accounts for 5 percent of soluble protein in acetate-grown cells. CODH purified from *M. barkeri* displays spectral characteristics similar to CO dehydrogenases purified from acetogens and is also a nickel-containing enzyme (*53*). The purified enzyme has a very high apparent Michaelis constant for CO (5 m*M*), indicating that CO is not the substrate of the enzyme in vivo. The catabolic function proposed for CODH is the oxidation of a carbonyl intermediate (the in vivo substrate) formed by cleavage of an acetyl moiety, which is then coupled with the reduction of methyl CoM to CH_4.

This model (Fig. 4) involves both membrane and soluble or cytoplasmic components. Baresi (*54*) described a membranous preparation from *M. barkeri* that produced methane under a nitrogen atmosphere. Recently, we demonstrated that methanogenesis from acetate occurred in a soluble protein fraction of *M. barkeri* when H_2 was present (*55*). Thus, we propose that electron transfer from CODH to methylreductase occurs with intact membranes. These two enzymes and others involved in the carbon transformation reactions of methanogenesis from acetate are cytoplasmic. They will produce methane from acetate, however, if the need for an electron transport chain is circumvented by exogenous hydrogen addition.

Several lines of evidence suggest that a redox mechanism and electron transport phosphorylation function in energy conservation during growth of *M. barkeri* on acetate (*14, 21, 52, 53, 55*): (i)



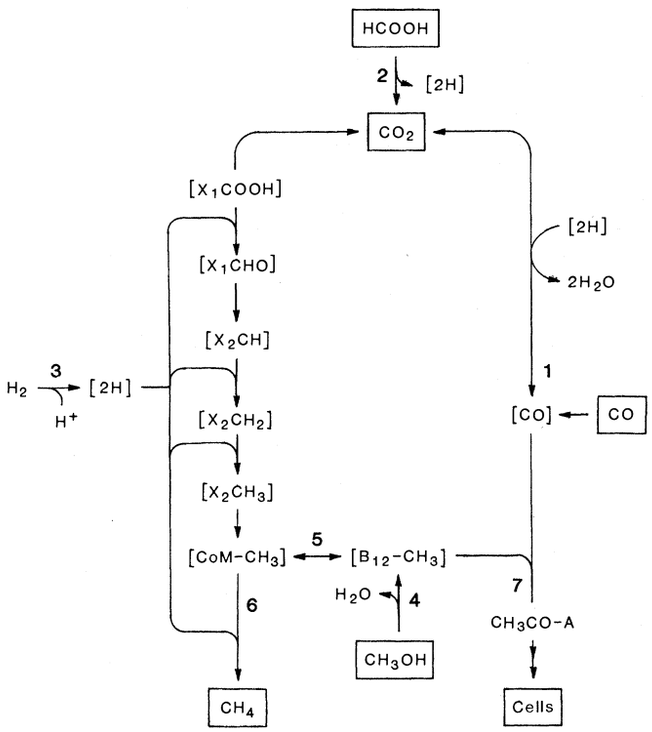


Fig. 2 (left). Model of carbon and electron flow proposed for energy conservation of acetogenic bacteria grown in the presence of CO. CO dehydrogenase (reaction 1) oxidizes 3CO to $3CO_2$ with the fourth CO becoming the acetate carboxyl precursor, [CO]. One CO_2 is reduced to methyl-tetrahydrofolate (CH_3-THF) by formate dehydrogenase (reaction 2), formyl-THF synthetase (reaction 3), methenyl-THF cyclohydrolase (reaction 4), methylene-THF dehydrogenase (reaction 5), and methylene-THF reductase (reaction 6). The methyl group is transferred to a corrinoid protein (X) and ultimately combined with [CO] to form acetyl-CoA (reaction 7). A transacetylase (reaction 8) and acetate kinase (reaction 9) are required for the final steps of acetate and ATP synthesis. Postulated electron carriers are indicated in their oxidized (A through F) and reduced (AH through FH) forms. This schematic representation does not indicate the exact configuration of electron transfer. Thus, whether AH reduces B or C directly or via intermediate carriers is unknown. The number which prefixes the electron carrier designation is the number of reducing equivalents required at each step, not the actual stoichiometry of electron or proton carriers involved. The carrier designated E must differ from D in that E must solely carry electrons, while D would carry both electrons and protons. Thus, the transfer of electrons from D to E would require the release of protons to the cell's exterior thereby generating an electrochemical gradient capable of synthesizing ATP via a proton-conducting membrane-bound adenosine triphosphatase (lower right-hand corner of the figure). Fig. 3 (right). Model of C_1 metabolism proposed for methanogenic bacteria that synthesize methane during growth on single carbon substrates or H_2 and CO_2. CO is oxidized to CO_2 by CO dehydrogenase (reaction 1), and formate is oxidized by formate dehydrogenase (reaction 2). The reducing equivalents (2H) are then used to reduce CO_2 to CH_4. Reducing equivalents from H_2 are derived via the action of hydrogenase (reaction 3). CO_2 is reduced to CH_4 through four redox states. CO_2 is activated on CDR factor (X_1), then reduced and transferred to the next carbon carrier, formaldehyde activating factor (X_2) or THMP. Further reduction takes place on THMP, perhaps to the methyl state where the C_1 unit is transferred to CH_3CoM. CH_3OH is transformed to methyl CoM via B_{12}–CH_3 by the action of methyltransferase I (reaction 4) and II (reaction 5). CH_3CoM is the direct precursor of methane and is acted on by methylreductase (reaction 6). Methyltransferase II may also be used to provide B_{12}–CH_3 from methanogenic intermediates for acetyl synthesis. [CO], which is derived by reduction of CO_2 via CO dehydrogenase, and B_{12}–CH_3 are condensed (reaction 7) to form an acetyl derivative (CH_3CO-A) which is the direct precursor for much of cell carbon synthesis. The synthesis of a methyl intermediate (a methane precursor or B_{12}–CH_3) links both catabolic and anabolic processes. No single methanogenic species can utilize all the substrates illustrated. *M. barkeri* catabolizes all the substrates except for formate.

higher levels of CODH, a reversible oxidoreductase, were found in acetate-grown cells as compared to cells grown in H_2 and CO_2; (ii) isotopic exchange and cyanide inhibition data indirectly suggest CODH involvement in acetate catabolism; (iii) methyl-CoM appears to be an intermediate of ^{14}C-2-acetate transformation by cell suspensions and thus electrons must be generated for methylreductase to form methane; (iv) trace amounts of H_2 are produced in association with methanogenesis from acetate; and (v) H_2 can replace the membrane requirement during methanogenesis from acetate in cell extracts. Membrane-bound cytochromes have been detected in acetate-grown *Methanosarcina* (*56*), although their function in coupling vectorial electron transfer to ATP synthesis has not been demonstrated.

The model proposed in Fig. 4 differs from others (*30, 57*) in that (i) it requires a redox process, (ii) methane is produced from acetate within the cell by soluble enzymes, and (iii) a carbonyl intermediate is the substrate of CODH in vivo, and is oxidized to provide electrons for reduction of methyl CoM to methane rather than methenyl THMP. The model is consistent with previous suggestions for the involvement of CODH in acetate catabolism (*14, 21, 52*). The possible use of a proton motive force to drive ATP synthesis in methanogenic bacteria has been reviewed (*29*).

Conclusions, Future Trends, and Biotechnological Applications

The biochemical analysis of these anaerobic bacteria and their enzyme components has extended our knowledge of biological systems, demonstrating unusual mechanisms by which anaerobes catabolize C_1 substrates and conserve energy. The taxonomically unrelated acetogens and methanogens have similar mechanisms for the interconversion of C_1 and C_2 compounds, with common enzyme activities (CODH) and chemically similar corrinoid proteins (methyl transferases) and pterin derivatives (THMP and THF) required by both groups for metabolic reactions. However, the mechanisms are biochemically distinct and many of the coenzymes of methanogenesis (such as F_{420}, F_{430}, and CoM) have not been found in acetogens.

At present, transformation of the C_1 substrates, formate, and acetic acid are of tremendous importance in waste treatment processes because methanogens can remove these metabolites, enabling mixed bacterial populations to effectively degrade organic matter to volatile products in anaerobic digestors (*58*). Further studies are needed to enhance the metabolic interaction of acetogens and methanogens with other trophic groups, and to develop defined and stable starter cultures of these bacteria for treatment of specific organic wastes.

Biomethanation to upgrade the thermal (Btu) content of coal gasification mixtures, and to remove toxic carbon monoxide via the biocatalytic application of methanogenic cells in high pressure reactors has also been considered (*59*).

Interest in acetic acid synthesis by acetogens has focused on synthesis from biomass-derived sugars (such as glucose) with high conversion efficiencies, and synthesis from C_1 compounds such as methanol and CO (*59, 60*). Advantages would include the less complicated technology required by the fermentation process as compared to current high pressure and temperature industrial processes, the possibility of recovering additional valuable products (such as vitamin B_{12}), and a reduced dependence on strategic minerals (such as rhodium). However, there are many economic and technological problems (*61*) including (i) the collection of suitable amounts of inexpensive substrates (such as cellulosic biomass), (ii) the demonstration of methods for recovery of dilute products and, (iii) the development of an economically competitive fermentation process given the existing capital investment and the efficiency of currently employed methods (>90 percent efficiency with the Monsanto process).

Thus, the knowledge of enzyme mechanisms (rather than the bacteria or their enzymes) may be useful in industrial organic acid or methane production, by leading to the use of synthetic or semisynthetic organometallic catalysts, based on their biological counterparts. Such systems would incorporate the mild conditions and selectivity generally attributed to biological catalysts, and simultaneously allow higher rates as well as higher product yield and concentration in accord with current industrial processes (*59*).

The biochemical cofactors and components present in these bacteria may also be of industrial interest. Methanol-grown

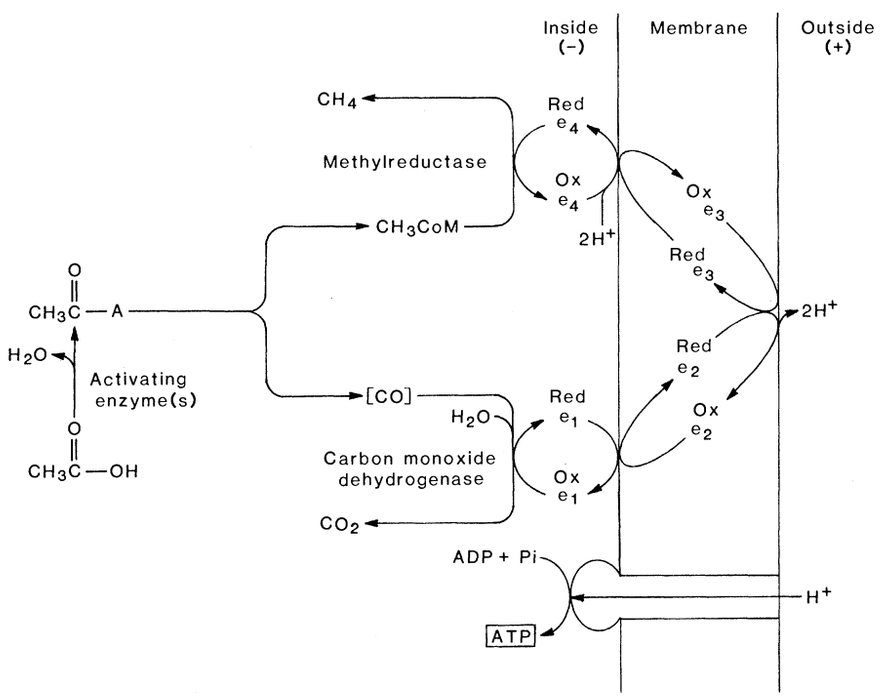


Fig. 4. Model of carbon and electron flow proposed for energy conservation of methanogenic bacteria grown on acetate. Acetate is transformed into an acetyl moiety which is then cleaved to yield a carbonyl [CO] and a methyl group; [CO] is oxidized to CO_2 by CO dehydrogenase. A soluble electron and proton carrier (e_1) is coupled to CO dehydrogenase and interacts with a membrane-bound electron and proton carrier (e_2). The proton carrier e_2 interacts with another membrane-bound component (e_3) which only carries electrons, and thus protons are released on the outside of the membrane. Reduced e_3 then transfers electrons to a soluble proton and electron carrier (e_4) which reduces CH_3CoM to methane via methylreductase. Protons for methyl CoM reduction are drawn from the cytoplasmic pool. The resultant proton gradient is used to drive ATP synthesis by membrane-bound adenosine triphosphatase. All carbon transformation reactions appear to be soluble since acetate transformation to methane readily occurs in the supernatant of 150,000*g* cell extract preparations supplemented with hydrogen. Only the electron transport chain (e_2 and e_3) need be integrally associated with the membrane.

acetogens and methanogens contain levels of B_{12}-like vitamins equivalent to those found in commercially used *Propionibacterium* species that are grown on more expensive substrates (*59*).

The biochemical components of these anaerobes may be of importance in understanding the diagenesis of petroleum (*62*). The ether-linked lipids of methanogens and other Archaebacteria may be microbial precursors of the isoprenoid hydrocarbons present in kerogen. Abelsonite is a nickel tetrapyrrole present in Green River shales (*63*). Although it was suggested that plant chlorophylls might be the precursor to abelsonite, this component of kerogen is structurally similar to the nickel tetrapyrrole, F_{430}, which appears to occur only in methanogens. Similarly, the high corrinoid contents of acetogens and methanogens could have also contributed as microbial precursors of kerogens which were naturally deposited by bacteria in anaerobic sedimentary environments.

Although the basic carbon flow to products in acetogenic and methanogenic bacteria is predictable, we will need to have a better understanding of the exact biochemistries (enzymes, coenzymes, electron carriers, and their cellular localization) in order to test the various proposed models for carbon and electron flow and energy conservation during growth on C_1 compounds. The isolation of metabolic mutants will aid in defining the specific biochemistry and the regulation of carbon and electron flow in these bacteria. Further study of the reaction centers of such metalloenzymes as CO dehydrogenase and their organometallic counterparts should prove mutually beneficial to chemists and biologists.

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