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Metabolism of homoacetogens

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Abstract

Homoacetogenic bacteria are strictly anaerobic microorganisms that catalyze the formation of acetate from C_1 units in their energy metabolism. Most of these organisms are able to grow at the expense of hydrogen plus CO_2 as the sole energy source. Hydrogen then serves as the electron donor for CO_2 reduction to acetate. The methyl group of acetate is formed from CO_2 via formate and reduced C_1 intermediates bound to tetrahydrofolate. The carboxyl group is derived from carbon monoxide, which is synthesized from CO_2 by carbon monoxide dehydrogenase. The latter enzyme also catalyzes the formation of acetyl-CoA from the methyl group plus CO. Acetyl-CoA is then converted either to acetate in the catabolism or to cell carbon in the anabolism of the bacteria. The homoacetogens are very versatile anaerobes, which convert a variety of different substrates to acetate as the major end product.

Introduction

Homoacetogenic bacteria, which are often also called 'acetogenic bacteria', are strictly anaerobic microorganisms, most of which catalyze the formation of acetate from H_2 plus CO_2 in their energy metabolism. The formation of acetate from H_2 plus CO_2 was first reported by Fischer et al. (1932) with enrichment cultures from sewage sludge. In addition, the same culture catalyzed the conversion of carbon monoxide to acetate. Wieringa (1936) succeeded in the purification of the acetogenic anaerobe *Clostridium aceticum* with H_2 plus CO_2 as substrates. This bacterium, however, soon was lost and was later reisolated by Adamse (1980) and by Braun et al. (1981). All attempts to isolate a chemolithotrophic acetogen with H_2 plus CO_2 failed until the purification of *Acetobacterium woodii* (Balch et al. 1977). In the meantime a further acetogen called *Clostridium thermoaceticum* was enriched with glucose as energy source at 60° C (Fontaine et al. 1942). The organism converted 1 glucose almost completely to near 3 acetate as end product. This new type of fermentation was therefore referred to as 'homoacetogenic' in analogy to the homofermentative lactate fermentation. The authors suggested either the cleavage of glucose to 3 C_2 units, which are converted to acetate, or the synthesis of one of the acetate molecules from two C_1 units. Fermentation of glucose in the presence of $^{14}CO_2$ yielded acetate equally labelled in both carbon atoms (Barker & Kamen 1945), supporting the idea that both C-atoms of acetate might originate from CO_2. Wood (1952) then demonstrated in a similar experiment, using $^{13}CO_2$ and mass analysis that part of the acetate contained two adjacent ^{13}C-atoms. This clearly indicated that both the carboxyl and the methyl group of a distinct acetate molecule could be derived from CO_2 (Fig. 1). Therefore, homoacetogenic bacteria can be defined as anaerobes catalyzing the formation of acetate (or higher fatty acids) from C_1 units (e.g. CO_2) in their energy metabolism.

Utilization of H_2 plus CO_2: the pathway of CO_2 reduction to acetate in homoacetogenic bacteria

Most homoacetogenic bacteria can grow chemolithoautotrophically on H_2 plus CO_2 as energy and cell carbon source, indicating that CO_2 reduction to acetate must be coupled with a net synthesis of ATP. The pathway

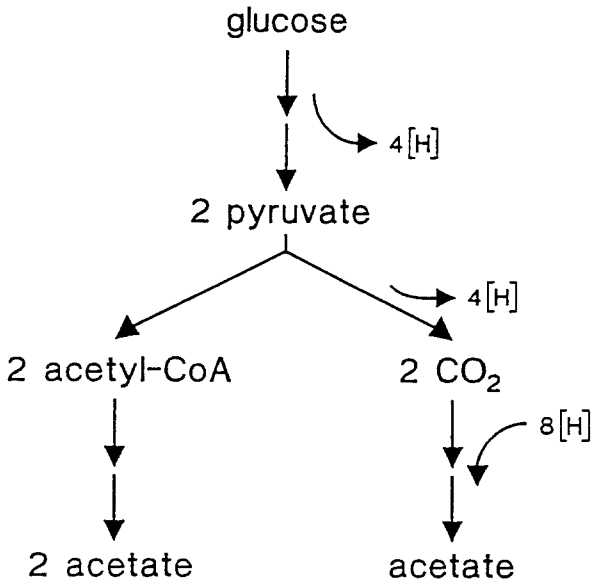

Fig. 1. Simplified tentative scheme of homoacetogenic fermentation of glucose. The tracer studies indicated that one of the three acetate formed is derived from CO_2.

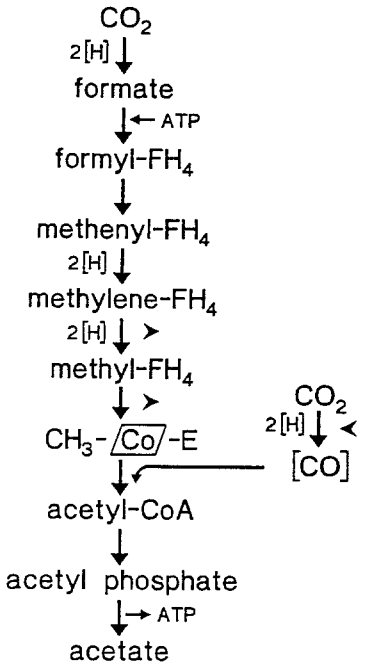

Fig. 2. Scheme of acetate synthesis from CO_2 in the energy metabolism of homoacetogenic bacteria. FH_4 = tetrahydrofolate, CH_3-Co-E = methylated corrinoid/Fe-S protein. Reactions coupled with energy conservation via a chemiosmotic mechanism are indicated by ▶, reactions requiring the input of energy by ◀.

of acetate formation from CO_2 was mainly elucidated with *Clostridium thermoaceticum* by the groups of H.G. Wood and of L.G. Ljungdahl (see also Wood 1991; Ljungdahl 1986). Since this pathway involves acetyl CoA as an intermediate and a carbon monoxide dehydrogenase as the key enzyme (see below), it is often referred to as 'acetyl CoA pathway' or as 'carbon monoxide dehydrogenase pathway'. The latter term will be used throughout this article. Acetate formation from H_2 plus CO_2 proceeds according to the following equation:

$$4\,H_2 + 2\,CO_2 \rightarrow \text{acetate}^- + H^+ + 2\,H_2O \quad (\Delta G^{o\prime} = -95\ \text{kJ/mol}) \qquad (1)$$

Synthesis of the methyl group of acetate from CO_2 was studied in detail in the 1960s (Ljungdahl & Wood 1969). C_1 units bound to tetrahydrofolate and a methyl group bound to a corrinoid have been shown to be intermediates in acetate formation (Ljungdahl et al. 1966; Poston et al. 1964). CO_2 is reduced to formate which is subsequently bound to tetrahydrofolate (FH_4). The formyl tetrahydrofolate (formyl-H_4folate = formyl-FH_4) is then reduced to methyl-H_4folate via methenyl- and methylene-H_4folate. The methyl group is transferred to a corrinoid protein, the methyl-corrinoid being the precursor of the methyl group of acetate.

The acetate synthesis from CO_2 is summarized in the scheme in Fig. 2.

Synthesis of the methyl group of acetate

The first step in the formation of the methyl group is the reduction of CO_2 to formate catalyzed by formate dehydrogenase:

$$CO_2 + 2\,[H] \rightarrow HCOO^- + H^+ \qquad (2)$$

The enzyme was purified from *C. thermoaceticum* (Andreesen & Ljungdahl 1974). The electron donor for the enzyme from *C. thermoaceticum* is NADPH; the $\Delta G^{o\prime}$ of the reaction is + 21.5 kJ/mol (Fuchs 1986). In other homoacetogens, e.g. *C. formicoaceticum*, the reductant is unknown. Since the enzyme from this organism catalyzes the reduction of methyl viologen as an artificial electron acceptor with formate as electron donor, it was postulated that ferredoxin (fd) might be the physiological electron donor (Ljungdahl & Wood 1982). Assuming a standard redox potential $E^{o\prime}$ of - 432 mV for the CO_2/formate couple (Thauer et al. 1977) and an $E^{o\prime}$ for fd_{ox}/fd_{red} of about - 350 mV

(*C. thermoaceticum*; Elliott & Ljungdahl 1982) or - 410 mV (*Peptostreptococcus productus*; Reubelt et al. 1991), the $\Delta G^{o\prime}$ of the formate dehydrogenase reaction would be + 15.8 or + 4.2 kJ/mol, respectively. The enzyme of *C. thermoaceticum* is oxygen-sensitive and contains selenium, present as selenocysteine, and tungsten, which probably replaces molybdenum found in other formate dehydrogenases. It is sensitive towards cyanide and azide.

Formate is bound to tetrahydrofolate (H_4folate) by the N^{10}-formyl tetrahydrofolate synthetase (Ljungdahl et al. 1970) at the expense of ATP:

$$\text{formate} + H_4\text{folate} + \text{ATP} \rightarrow N^{10}\text{-formyl-}H_4\text{folate} + \text{ADP} + P_i \quad (3)$$

The $\Delta G^{o\prime}$-value for this reaction is - 8.4 kJ/mol. Formate has to be activated prior to further reduction to the level of formaldehyde, since the reduction of a carboxylic acid to the corresponding aldehyde is a highly endergonic reaction (Thauer et al. 1977). The following step, the dehydration of formyl-H_4folate, is mediated by the enzyme N^5, N^{10}-methenyl tetrahydrofolate cyclohydrolase (O'Brien et al. 1973):

$$N^{10}\text{-formyl-}H_4\text{folate} \rightarrow N^5, N^{10}\text{-methenyl-}H_4\text{folate}^+ + OH^- \quad (4)$$
$$(\Delta G^{o\prime} = -4.0 \text{ kJ/mol})$$

Methenyl tetrahydrofolate is then reduced to methylene-H_4folate by N^5, N^{10}-methylene tetrahydrofolate dehydrogenase (O'Brien et al. 1973):

$$N^5, N^{10}\text{-methenyl-}H_4\text{folate}^+ + \text{NAD(P)H} \rightarrow N^5, N^{10}\text{-methylene-}H_4\text{folate} + \text{NAD(P)}^+ \quad (5)$$

The $\Delta G^{o\prime}$ is - 4.9 kJ/mol (Fuchs 1986). In *C. thermoaceticum* the reaction is specific for NADPH, in *C. formicoaceticum* for NADH (Moore et al. 1974). The enzyme in *C. thermoaceticum* is bifunctional and also catalyzes the cyclohydrolase reaction (4). In the subsequent reaction methylene tetrahydrofolate is reduced to a bound methanol by N^5, N^{10}-methylene tetrahydrofolate reductase, one of the most important enzymes of the homoacetogenic pathway:

$$N^5, N^{10}\text{-methylene-}H_4\text{folate} + 2\,[H] \rightarrow N^5\text{-methyl-}H_4\text{folate} \quad (6)$$

For the enzyme of most homoacetogens investigated so far, ferredoxin has been proposed to be the physiological reductant (Clark & Ljungdahl 1984; Ljungdahl 1986). Recently the standard redox potential $E^{o\prime}$ of the couple methylene- /methyl-H_4folate has been determined to be near - 200 mV (Wohlfarth & Diekert 1991). Provided an $E^{o\prime}$ of ferredoxin of - 350 (see above, reaction (2)) the $\Delta G^{o\prime}$ of reaction (6) is - 29.0 kJ/mol. In the carbon monoxide-utilizing homoacetogen *Peptostreptococcus productus* the reducing equivalents for the methylene-H_4folate reduction are supplied by NADH ($E^{o\prime}$ = - 320 mV) rather than ferredoxin (Wohlfarth et al. 1990); the $\Delta G^{o\prime}$ can be calculated to be - 22.0 kJ/mol. Therefore, even with NADH as the electron donor, the free energy of the methylene tetrahydrofolate reduction is sufficiently exergonic to account for the synthesis of at least one third of an ATP by a chemiosmotic mechanism (Diekert & Wohlfarth 1994a). Until now, it is still unclear whether this reaction is involved in energy conservation of homoacetogenic bacteria. Evidence has been presented for *Clostridium thermoautotrophicum* (Hugenholtz et al. 1987) and for *P. productus* (Wohlfarth et al. 1990), that the enzyme might be membrane associated – a prerequisite for energy coupling via a chemiosmotic mechanism.

The methyl group of methyl tetrahydrofolate is subsequently transferred to a corrinoid/iron-sulfur protein (E-[Co]) (Ragsdale et al. 1987) where it is bound to the cobalt of the cob(I)amide prosthetic group by a methyl transferase (Drake et al. 1981):

$$N^5\text{-methyl-}H_4\text{folate} + \text{E-[Co]} \rightarrow H_4\text{folate} + \text{E-[Co]-CH}_3 \quad (7)$$

Formally, the methyl group is transferred as a methyl$^+$ (i.e. a bound methanol) to Cobalt(I) of the cobamide. The methyl transferase purified from *C. thermoaceticum* (Drake et al. 1981) is a homodimer without striking characteristics. The corrinoid/Fe-S protein was also purified from *C. thermoaceticum* (Ragsdale et al. 1987). It is a heterodimer containing a [4 Fe-4 S] cluster and a 5′-methoxybenzimidazolylcobamide. Like most B_{12} proteins also the corrinoid/Fe-S protein is sensitive towards alkyl halides such as propyl iodide. The methyl group bound to the cobamide is the methyl donor for acetyl-CoA synthesis.

Synthesis of the carboxyl group of acetate

The methyl group of acetate is provided by a bound methanol. This implies that for carboxyl group formation, CO_2 has to be reduced prior to its incorporation into acetate. For a long time the synthesis of the carboxyl group was enigmatic. Schulman et al. (1973) concluded from tracer experiments that the carboxyl of acetate during fermentation of pyruvate by cell extracts of *C. thermoaceticum* was derived from C_1 of pyruvate rather than from free CO_2. This conclusion was mainly derived from the findings that i) the synthesis of acetate in cell extracts was strictly dependent on pyruvate, which could not be replaced by CO_2 plus reducing equivalents, and ii) the specific radioactivity of acetate during the incubation always paralleled that of C_1 of the α-ketoacid rather than of CO_2. This indicates, that – at least under certain experimental conditions – the carboxyl of acetate could be directly formed from C_1 of pyruvate.

In the chemolithotrophic homoacetogens able to grow with H_2 plus CO_2 the carboxyl group of acetate has to be formed from CO_2 rather than from organic compounds. A crucial step in the elucidation of the carboxyl synthesis from CO_2 was the discovery of high activities of carbon monoxide dehydrogenase in homoacetogens (Diekert & Thauer 1978). This enzyme *in vitro* mediates the reduction of methyl viologen (MV) with CO; the physiological electron acceptor (or donor in the reverse reaction) is probably ferredoxin (Ragsdale et al. 1983a). *In vivo* the carbon monoxide dehydrogenase reaction in the synthesis of acetate from CO_2 is the following (Diekert et al. 1984):

$$CO_2 + 2\,[H] \rightarrow [CO] + H_2O \qquad (8)$$

where [CO] indicates carbon monoxide in an enzyme-bound form. With 2 [H] = reduced ferredoxin the standard free energy $\Delta G^{o\prime}$ ranges between + 22.0 and + 33.5 kJ/mol corresponding to a redox potential of ferredoxin between - 350 and - 410 mV (see above) and assuming an $E^{o\prime}$ for CO_2/CO of - 524 mV (Fuchs 1986). The carbon monoxide dehydrogenase measured by methyl viologen reduction with CO is sensitive against cyanide and in most cases also extremely oxygen sensitive. Evidence for the physiological role of the CO dehydrogenase in CO_2 reduction to CO (in a bound form) was derived from the finding, that i) homoacetogens produced traces of carbon monoxide; ii) CO formation was sensitive towards cyanide, and iii) CO formation was stimulated by propyl iodide (Diekert et al. 1984). The latter effect was probably due to alkylation of the corrinoid/Fe-S protein (see above), which no longer could serve as the methyl carrier in acetate formation, leading to the accumulation of CO as intermediate in the carboxyl group synthesis. The fact that only very low amounts of CO are formed during acetate formation from CO_2 indicates that the product of reaction (8) is an enzyme-bound carbonyl rather than free CO.

The carbon monoxide dehydrogenase is a bifunctional enzyme, which, in addition to CO_2 reduction to CO, mediates the subsequent reaction, i.e. the formation of acetyl-CoA from the bound methanol (E-[Co]-CH_3) plus the bound CO (Roberts et al. 1992):

$$\text{E-[Co]-CH}_3 + [CO] + \text{HS-CoA} \rightarrow \text{E-[Co]} + \text{acetyl-CoA} \qquad (9)$$

The $\Delta G^{o\prime}$ of this reaction is unknown (see below). Evidence for the physiological function of the carbon monoxide dehydrogenase in acetyl-CoA synthesis was provided by the finding that the enzyme purified from *C. thermoaceticum* mediated the isotopic exchange between acetyl-CoA labelled in C_1 and unlabelled CO (Ragsdale et al. 1983a). Acetyl-CoA is further converted to acetate via acetyl phosphate as the intermediate. The enzymes involved are phosphate acetyltransferase (phosphotransacetylase) and acetate kinase:

$$\text{acetyl-CoA} + P_i \rightarrow \text{acetyl phosphate} + \text{HS-CoA} \qquad (\Delta G^{o\prime} = +\ 9.0\ \text{kJ/mol}) \qquad (10)$$

$$\text{acetyl phosphate} + \text{ADP} \rightarrow \text{acetate} + \text{ATP} \qquad (\Delta G^{o\prime} = -\ 13.0\ \text{kJ/mol}) \qquad (11)$$

In the anabolism of homoacetogenic bacteria (and other anaerobes; see below) the carbon monoxide pathway also serves biosynthetic purposes as an autotrophic CO_2 fixation pathway. Therefore, a minor part of acetyl-CoA is reductively carboxylated to pyruvate by pyruvate:ferredoxin oxidoreductase; pyruvate is then converted to cell compounds via phosphoenolpyruvate as intermediate (Fuchs 1986). Acetate synthesis from CO_2 is summarized in the scheme shown in Fig. 2 (see also Fuchs 1986; Ragsdale 1991; Diekert 1992).

Energetics of the carbon monoxide dehydrogenase pathway

The carbon monoxide dehydrogenase pathway of acetate synthesis has to be coupled with a net synthesis of ATP. It is still unclear which reaction(s) is

(are) involved in energy conservation. For activation of formate one ATP is consumed. In the acetate kinase reaction one ATP is stoichiometrically synthesized per acetate formed. Since part of the acetyl-CoA is converted to cell carbon and therefore is not available for ATP synthesis, other reactions in the carbon monoxide dehydrogenase pathway have to be coupled to ATP synthesis. Since none of these reactions can mechanistically be coupled to substrate level phosphorylation, energy has to be conserved by a chemiosmotic mechanism. Moreover, evidence has been presented that the CO dehydrogenase reaction (8), i.e. the formation of CO from CO_2 is an endergonic reaction that is most probably driven by an electrochemical proton gradient (Diekert et al. 1986). The $\Delta G^{o\prime}$ of this reaction acounts for 1 electrogenic proton translocated corresponding to approximately $^1/_3$ ATP consumed per CO formed. The reactions possibly involved in energy conservation are reactions (6) and (7), i.e. methylene-H_4folate reduction to methyl tetrahydrofolate and methyltransfer from H_4folate to the corrinoid/Fe-S protein. As stated above, methylene tetrahydrofolate reduction could be coupled to the synthesis of $^1/_3$ to $^2/_3$ ATP (Diekert & Wohlfarth 1994a). The $\Delta G^{o\prime}$ of the methyl transferase reaction is not known; however, the $\Delta G^{o\prime}$ of acetyl-CoA formation from CO and methyl tetrahydrofolate (reactions (7) plus (9)) can be calculated to be - 38.0 kJ/mol (Diekert & Wohlfarth 1994a), thus accounting for additional $^1/_3$ to $^2/_3$ ATP synthesized per acetyl-CoA produced. It appears unlikely that the acetyl-CoA formation from the corrinoid-bound methyl group and the enzyme-bound CO is an energy conserving reaction since acetyl-CoA as the reaction product is an energy-rich compound the formation of which should not be sufficiently exergonic. Therefore, also supported by the analogy to the methyl transferase reaction in methanogenic bacteria (Becher et al. 1992), the involvement of methyl transferase in ATP synthesis is by far more feasible. A methyl transfer coupled to an electrogenic cation translocation represents a new type of energy conserving reaction.

Recently it was discovered with *P. productus* that acetate formation from CO_2 was dependent on sodium (Geerligs et al. 1989). This was later confirmed for *Acetobacterium woodii* (Heise et al. 1989) and *Acetogenium kivui* (Yang & Drake 1990). With inverted vesicles prepared from *A. woodii* it could be demonstrated that a sodium gradient was built up upon acetate formation from CO in the absence and presence of formaldehyde (Heise et al. 1993), indicating that at least one of the reactions proposed to be coupled to ATP synthesis might conserve energy via an electrochemical sodium gradient rather than a proton gradient (see also Diekert & Wohlfarth 1994a). Evidence is available that the ATP synthase in *A. woodii* is a sodium translocating ATPase (Heise et al. 1992). It cannot be excluded, however, that other homoacetogenic bacteria involve a proton gradient rather than a sodium gradient in energy conservation. A tentative scheme of ATP synthesis coupled to acetate formation from H_2 plus CO_2 via a sodium gradient is shown in Fig. 3.

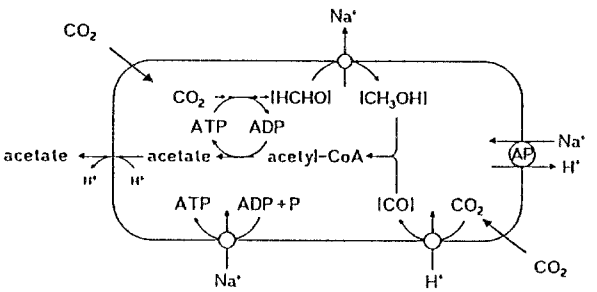

Fig. 3. Energy conservation coupled with acetate formation from CO_2 via an electrochemical sodium gradient. [HCHO] = bound formaldehyde = methylene-FH_4; [CH_3OH] = bound methanol = methyl-corrinoid/Fe-S protein; AP = sodium/proton antiporter. Note, that no stoichiometries of monovalent cations translocated across the membrane are given here.

Carbon monoxide dehydrogenase: a key enzyme in homoacetogenesis

Due to its bifunctionality, carbon monoxide dehydrogenase can be considered to be the key enzyme in this pathway, which is therefore often designated 'carbon monoxide dehydrogenase pathway'. In addition, the enzyme exhibits some interesting structural features and a unique reaction mechanism. One of the most outstanding properties of the enzyme is that it contains nickel as a cofactor. The nickel dependence of the synthesis of CO dehydrogenase has been discovered in a non-homoacetogenic bacterium, namely *Clostridium pasteurianum* (Diekert et al. 1979), and was later confirmed for the homoacetogens *C. thermoaceticum* and *C. formicoaceticum* (Diekert & Thauer 1980). In the meantime, the enzyme has been purified from several homoacetogenic (Diekert & Ritter 1983; Ragsdale et al. 1983a, 1983b) and other anaerobic, non-homoacetogenic bacteria (see also Diekert & Wohlfarth 1994b). Besides nickel, the enzyme also contains iron and acid-labile sulfur. Evi-

dence was presented for the enzyme from *C. thermoaceticum* that at least part of the nickel is bound close to iron and sulfur (Bastian et al. 1988) and that the carbon of CO binds to nickel (Ragsdale et al. 1985). Two different sites for CO oxidation and acetyl-CoA synthesis could be distinguished (Shin et al. 1993; Kumar et al. 1993), which is supported by the finding that CO oxidation rather than acetyl-CoA formation was sensitive towards cyanide (Diekert et al. 1984). Possibly both sites contain nickel plus Fe/S.

Whereas the CO oxidation mediated by CO dehydrogenase can be easily quantitated by methyl viologen reduction with CO, the measurement of acetyl-CoA formation from methyl-H_4folate and CO requires several proteins (Roberts et al. 1992), namely carbon monoxide dehydrogenase, methyl transferase, the corrinoid/Fe-S protein, and ferredoxin II from *C. thermoaceticum*. Ferredoxin is not necessarily required and has a stimulating effect in this system. A role of ferredoxin in the activation of CO dehydrogenase and the corrinoid/Fe-S protein has been proposed (Roberts et al. 1992). The hypothetical reaction mechanism of acetyl-CoA formation is summarized in Fig. 4.

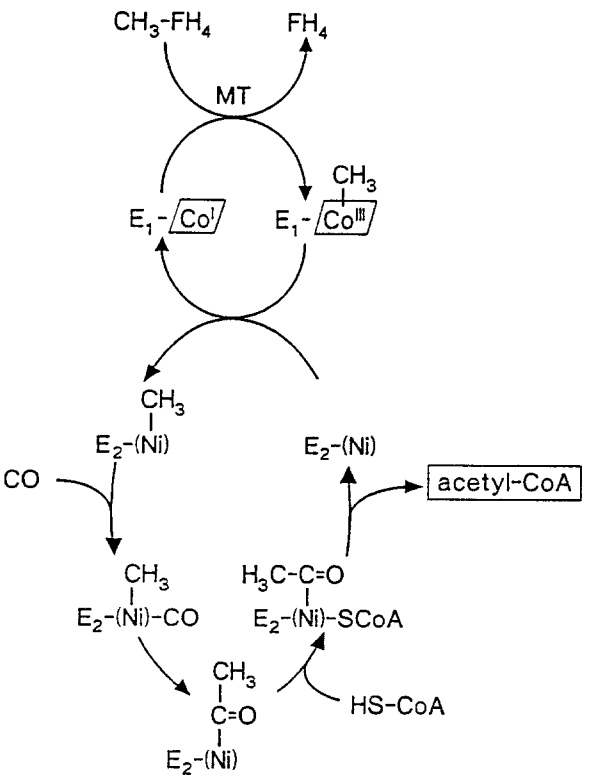

Fig. 4. Tentative scheme of the mechanism of acetyl-CoA formation from CO and methyl tetrahydrofolate according to Roberts et al. (1992). E_1 = corrinoid/Fe-S protein; E_2 = carbon monoxide dehydrogenase; MT = methyl transferase; FH_4 = tetrahydrofolate.

Utilization of other C_1 substrates

Carbon monoxide and formate

Carbon monoxide as well as formate are intermediates in acetate formation from CO_2. Therefore, it is not surprising that some homoacetogenic bacteria are able to grow at the expense of formate or carbon monoxide as energy substrates. Formate is oxidized to CO_2, and the reducing equivalents derived from formate oxidation are utilized for the reduction of formate (or formyl-H_4folate) to bound methanol and of CO_2 to CO. The bound carbonyl and the methyl group are then combined to yield acetyl-CoA.

Carbon monoxide is readily utilized only by a few homoacetogenic bacteria, e.g. *P. productus* (Lorowitz & Bryant 1984; Geerligs et al. 1987) or *Eubacterium limosum* (Genthner & Bryant 1982). Acetate is formed from CO according to the following equation:

$$4\ CO + 2\ H_2O \rightarrow acetate^- + H^+ + 2\ CO_2 \quad (\Delta G^{o\prime} = -165.6\ kJ/mol) \qquad (12)$$

In contrast to formate, carbon monoxide is not a *free* intermediate in acetate synthesis. Therefore, two possible mechanisms of CO conversion to acetate are feasible: i) Carbon monoxide serves exclusively as electron donor for the reduction of CO_2 to acetate, i.e. 4 CO are oxidized to 4 CO_2, and 2 CO_2 are reduced to acetate according to the pathway described above; ii) carbon monoxide is a reductant and a direct precursor for the synthesis of the carboxyl group of acetate, i.e. 3 CO are converted to 3 CO_2 plus 6 [H], the latter being required for the synthesis of the methyl group from CO_2, and the methyl group is combined with a fourth CO to acetyl-CoA. The main difference between the two possibilities consists in involving CO oxidation to CO_2 prior to CO_2 reduction to a bound carbonyl as the precursor of the carboxyl group of acetate according to mechanism i) or in the 'direct' incorporation of free CO into the carboxyl group according to mechanism ii). Evidence is available for *P. productus* that carbon monoxide is directly incorporated into C_1 of acetate, whereas other homoacetogenic bacteria, which are not capable of fast growth with CO, oxidize carbon monoxide to CO_2 and reduce CO_2 to a bound carbonyl as the precursor for acetate synthesis (Ma et al. 1987). This

discrepancy might explain why *P. productus* is able to grow fast with CO as the energy source. The reason for this difference is still unclear. It could be due to an inhibition of acetate synthesis by higher CO concentrations in non-CO-utilizing homoacetogens or to a difference in the reaction mechanism of the CO dehydrogenases.

Carbon monoxide can be converted by several homoacetogens to reaction products other than acetate, for example ethanol (Tanner et al. 1993) or butanol (Grethlein et al. 1991). This might be of interest for biotechnological solvent formation from inexpensive industrial products.

Carboxyl sources other than CO_2 might serve the homoacetogens as electron acceptors for metabolic oxidation reactions. *C. thermoaceticum* utilizes the carboxyl group of substituted benzoates after decarboxylation to CO_2 as an electron sink for the oxidation of methyl substrates (Hsu et al. 1990).

Methyl compounds

Several homoacetogens grow with methyl compounds as energy source. The most important methyl substrates are methanol (Hamlett & Blaylock 1969; Zeikus et al. 1980), methoxylated aromatic compounds such as vanillate or syringate (Bache & Pfennig 1981), and methyl chloride (Traunecker et al. 1991). Methyl compounds (CH_3-X) are converted to acetate according to the following equation:

$$4\ CH_3\text{-}X + 2\ CO_2 + 2\ H_2O \rightarrow 3\ acetate^- + 7\ H^+ + 4\ X^- \qquad (13)$$

The $\Delta G^{o\prime}$-value for CH_3-X = CH_3OH is - 71.0 kJ/mol, for CH_3Cl - 140.0 kJ/mol. *Butyribacterium methylotrophicum* (Zeikus et al. 1980), *Eubacterium limosum* (Genthner et al. 1981), *A. woodii* (Bache & Pfennig 1981), and *Sporomusa* spec. (Breznak 1992) are well-studied methylotrophic homoacetogens. Usually, methylotrophic homoacetogens can utilize both methanol or methoxylated aromatic compounds; however, generally only the methoxyl group of the latter substrate rather than the aromatic nucleus is utilized. Until now, only one methylotrophic homoacetogen (designated strain MC) has been described that is also able to grow at the expense of methyl chloride as the energy source (Traunecker et al. 1991).

Evidence is available that methanol utilization by *Clostridium thermoautotrophicum* involves a methanol dehydrogenase containing pyrrolo quinoline quinone (PQQ) and mediating the oxidation of methanol via formaldehyde to formate (Winters & Ljungdahl 1989). However, it is doubtful whether this is a general principle in methanol-utilizing homoacetogens. In other methylotrophic homoacetogens the methyl group of methanol is probably transferred to a methyl acceptor in the CO dehydrogenase pathway (van der Meijden et al. 1984).

There are two possible acceptors for the methyl group in the carbon monoxide dehydrogenase pathway, namely the corrinoid/Fe-S protein or tetrahydrofolate. It is of great importance for energy conservation whether the methyl group is transferred to H_4folate or the corrinoid protein, due to the direction of the methyl transferase reaction. This reaction, dependent on its direction, most likely either requires the input of metabolic energy or is coupled with ATP synthesis via a chemiosmotic mechanism (see above). With tetrahydrofolate as the primary methyl acceptor, three methyl groups per three acetate formed have to be transferred by the methyl transferase to the corrinoid protein in an exergonic reaction, and one methyl group has to be oxidized to CO_2 thus providing the reducing equivalents for the reduction of 3 CO_2 to 3 carbonyls as the precursor of C_1 of acetate. If the methyl group is directly transferred to the corrinoid protein the exergonic methyl transferase reaction would be circumvented; instead, one methyl group is oxidized to CO_2 per 3 acetate formed involving the methyl transferase in the endergonic direction (Fig. 5). Higher growth yields obtained with phenylmethyl-ethers or methyl chloride as compared to those with methanol may be interpreted to indicate that the methyl group of methanol is transferred to the corrinoid protein whereas the acceptor for the methyl groups of methoxylated aromatic compounds or methyl chloride is tetrahydrofolate (Tschech & Pfennig 1984; Daniel et al. 1991; Berman & Frazer 1992; Traunecker et al. 1991), according to the more favourable pathway of methyl chloride or methoxyl group conversion to acetate (Diekert & Wohlfarth 1994a).

Cell-free extracts of the methyl chloride-utilizing strain MC mediated the formation of methyl tetrahydrofolate from tetrahydrofolate plus methyl chloride or vanillate, respectively (Meßmer et al. 1993). The reaction proceeded quantitatively and stoichiometrically, indicating that the methyl transferase reaction in the direction of the endergonic methyl transfer from the corrinoid protein to tetrahydrofolate is not involved. This further supports the assumption that the methyl group of methyl chloride or phenylmethylethers such

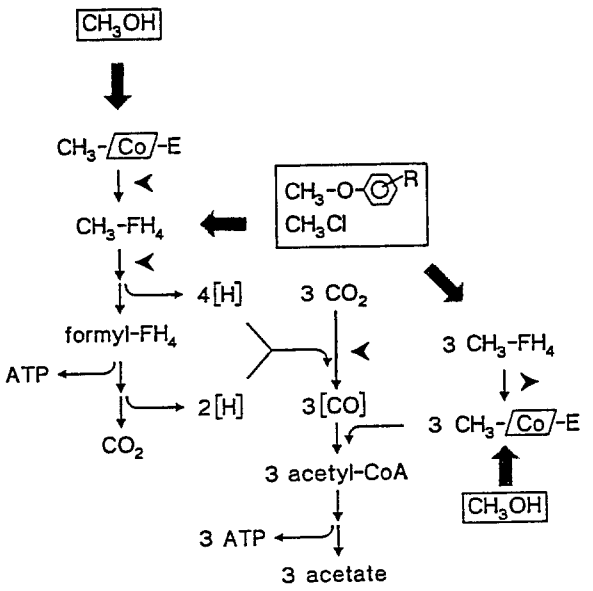

Fig. 5. Tentative scheme of acetate formation from methyl compounds such as methanol, phenylmethylethers, and methyl chloride. Reactions requiring the input of energy via a chemiosmotic mechanism are indicated by ◀, reactions coupled with energy conservation by ▶. FH_4 = tetrahydrofolate, CH_3-Co-E = methylated corrinoid/Fe-S protein. The thick grey arrows indicate, where the methyl compounds presumably enter the carbon monoxide dehydrogenase pathway.

as vanillate is directly transferred to tetrahydrofolate rather than via the corrinoid protein. The methylation of a corrinoid protein by 3,4-dimethoxybenzoate observed in cell-free extracts of *Sporomusa ovata* (Stupperich & Konle 1993) could be due to the formation of methyl tetrahydrofolate followed by the subsequent exergonic transfer of the methyl group from tetrahydrofolate to the corrinoid. With strain TMBS4, a homoacetogen growing with trimethoxybenzoate, it was shown that the CO_2-dependent O-demethylation of trimethoxybenzoate was light-reversibly inactivated by propyl iodide. This indicated that a corrinoid might be involved in acetate formation (or CO_2 formation) from the methoxyl group (Kreft & Schink 1993). The effect of propyl iodide is probably due to its reaction with the corrinoid/iron-sulfur protein involved in acetate formation from methyl substrates.

Wherever the methyl group enters the carbon monoxide dehydrogenase pathway, part of it has to be oxidized to CO_2 (see also Fig. 5). Therefore, methyl tetrahydrofolate is oxidized to methylene tetrahydrofolate. Provided that the methylene tetrahydrofolate reductase plays a role in energy conservation via a chemiosmotic mechanism, it must be assumed that the methyl tetrahydrofolate oxidation is either driven by an electrochemical ion gradient or that an electron acceptor with a more positive redox potential than that of ferredoxin (or possibly pyridine nucleotides) is involved.

Several homoacetogens, but not all, have been reported to contain cytochromes (b- and/or c-type) and quinones (Gottwald et al. 1975). The role of these electron carriers in homoacetogens is not obvious. It has been proposed that a cytochrome might serve as electron acceptor for methyl oxidation (Hugenholtz & Ljungdahl 1990; Kamlage et al. 1993; Kamlage & Blaut 1993). It should, however, be mentioned that for instance *A. woodii*, which has been the first homoacetogen reported to utilize methoxylated aromatic compounds, obviously does not contain cytochromes or quinones (see Diekert 1992). If cytochromes play a role in methanol or methyl compounds oxidation, this is apparently not valid for all homoacetogens. In addition, a function of cytochromes in acetogenesis from CO_2 has been discussed (Hugenholtz & Ljungdahl 1990). The same objections mentioned above for the role of cytochromes in the oxidation of methyl compounds is valid for their function in CO_2 reduction to acetate: there are several homoacetogens without any detectable cytochromes and quinones that are able to reduce CO_2 to acetate. Therefore, the role of cytochromes and/or quinones in homoacetogenesis is still enigmatic. In fumarate-reducing homoacetogens such as *Clostridium formicoaceticum*, cytochrome b and menaquinone most probably are involved in fumarate reduction (Dorn et al. 1978), as it is well-known for other fumarate-reducing anaerobes (Kröger et al. 1992).

A few homoacetogenic bacteria may channel the methyl group of methoxylated aromatic substrates into other pathways. Strain TMBS4 has been reported to transfer the methyl groups of trimethoxybenzoate to sulfide upon formation of dimethylsulfide or, to a less extent, methanethiol (Bak et al. 1992). The aromatic nucleus of the product trihydroxybenzoate is further degraded to acetate via the phloroglucinol pathway (Kreft & Schink 1993). It cannot be excluded that the formation of dimethylsulfide is an unphysiological reaction (Bak et al. 1992). At least the major part, if not all, of the energy generated by trimethoxybenzoate degradation with sulfide is derived from the degradation of the aromatic ring system to acetate. No evidence is available that the methyl group transfer from the

methoxyl compound to sulfide is coupled with energy conservation.

Recently, it was found that *C. thermoaceticum* mediates the oxidation of the methyl group of vanillate (4-hydroxy-3-methoxybenzoate) with nitrate as terminal electron acceptor (Seifritz et al. 1993), thus confirming the versatility and metabolic potential of homoacetogens. Alternatively, *C. formicoaceticum* is able to transfer the reducing equivalents generated upon methyl oxidation to fumarate (Matthies et al. 1993).

Other methyl or methoxyl compounds such as betaine (Möller et al. 1984), methoxyacetate (Schuppert & Schink 1990), and methoxyethanol (Tanaka & Pfennig 1988) are probably converted to acetate in a pathway similar to that described above for the O-demethylating CO dehydrogenase pathway.

Utilization of alternative substrates and electron acceptors

Two-carbon substrates

Several homoacetogens are able to utilize reduced two-carbon substrates or their derivatives such as ethanol (Eichler & Schink 1984), 2-ethoxyethanol (Tanaka & Pfennig 1988), ethyleneglycol (Eichler & Schink 1984) or polyethyleneglycol (Wagener & Schink 1988), oxalate, or glyoxylate (Daniel & Drake 1993). Ethanol is oxidized to acetate, the reducing equivalents being transferred to CO_2 upon formation of acetate via the CO dehydrogenase pathway. The utilization of polyethyleneglycol and ethyleneglycol (Schramm & Schink 1991) and probably also of 2-ethoxyethanol (Tanaka & Pfennig 1988) is initiated by a C_1-C_2-shift of the OH-group followed by splitting off acetaldehyde (or formation of acetaldehyde, respectively), which is oxidized to acetate. The reducing equivalents can either be transferred to CO_2 upon formation of acetate via the CO dehydrogenase pathway or, upon utilization of polyethyleneglycol, the formation of propionate from acetaldehyde via methylmalonyl-CoA as intermediate might serve as an electron sink for acetaldehyde oxidation (Wagener & Schink 1988). Oxalate and glyoxylate are converted to acetate plus CO_2 by *C. thermoaceticum* via still unknown pathways (Daniel & Drake 1993).

A thermophilic homoacetogenic bacterium tentatively called strain AOR or 'Reversibacterium' has been reported to grow with acetate as sole energy source under extremely low H_2-partial pressure ($\leqq 10^{-6}$ bar) (Lee & Zinder 1988a). The organism converts acetate to H_2 plus CO_2 via a 'reversed carbon monoxide dehydrogenase pathway' (Lee & Zinder 1988b). The organism is also able to grow under 'laboratory conditions' (i.e. $[H_2] \approx 1$ bar) with H_2 plus CO_2. This demonstrates the ability of homoacetogens to adapt to the environmental conditions. At low H_2 concentrations the oxidation of acetate to CO_2 with protons as electron acceptor is exergonic, at high hydrogen partial pressures the formation of acetate from H_2 plus CO_2 is thermodynamically favourable (see reaction (1) and Diekert 1992). It is feasible that acetate conversion to H_2 plus CO_2 involves electron carriers different from those involved in acetate synthesis from CO_2 plus H_2.

Alternative substrates or electron acceptors

Fumarate (Dorn et al. 1978; Plugge et al. 1993; Matthies et al. 1993) or nitrate (Seifritz et al. 1993) have already been mentioned to serve as external alternative electron acceptors for catabolic oxidation reactions in several homoacetogens (see above). The reduction of these compounds is most probably coupled to the synthesis of ATP via a chemiosmotic mechanism. Homoacetogens like *C. formicoaceticum* can grow at the expense of fumarate as *sole* energy source. The substrate is oxidized either completely to CO_2 involving the carbon monoxide dehydrogenase pathway (Plugge et al. 1993) or incompletely to acetate (Dorn et al. 1978), using fumarate reduction to succinate as electron sink. Other unsaturated organic compounds may serve several homoacetogens as alternative electron acceptors for the oxidation of reduced substrates. *A. woodii* has been reported to utilize the propenoate side chain of caffeate (3-(3,4-dihydroxyphenyl)-2-propenoate) as an electron sink (Tschech & Pfennig 1984). Evidence is available that this reduction is coupled to the formation of ATP (Hansen et al. 1988).

Homoacetogenic fermentation of sugars in cyanobacteria

Recently the anaerobic homoacetogenic fermentation of carbohydrates in the dark has been reported for a strain of the cyanobacterium *Oscillatoria limosa* (Heyer et al. 1989) and for *Nostoc sp.* strain Cc (Margheri & Allotta 1993). In *Oscillatoria* the presence of a carbon

monoxide dehydrogenase has been demonstrated. In *Nostoc* the fermentation obviously supported survival rather than growth of the bacteria. It is feasible that the formation of the 'third' acetate from hexose proceeds via the carbon monoxide dehydrogenase pathway. The function of this fermentation in cyanobacteria could be to support the organisms in the absence of oxygen in the dark, e.g. on the sediment surface of a shallow eutrophic pond.

Related pathways involving anaerobic carbon monoxide dehydrogenase

It has already been mentioned that the carbon monoxide dehydrogenase pathway also serves biosynthetic purposes as autotrophic CO_2 fixation pathway in several anaerobes (see above). Besides homoacetogens, also chemolithoautotrophic methanogens (see chapter M. Blaut) and sulfidogens (see chapter T. Hansen) catalyze the reduction of CO_2 to acetyl-CoA as the first CO_2 fixation product. Acetyl-CoA is reductively carboxylated to pyruvate, which is subsequently further converted via phosphoenolpyruvate to triosephosphate (Fuchs 1986; Diekert 1992). In methanogenic bacteria autotrophic CO_2 fixation involves coenzymes specific for methanogens rather than the coenzymes of homoacetogens or sulfidogens (Whitman et al. 1992).

The so-called 'acetyl-CoA pathway' is probably the most widely used CO_2 fixation pathway under anoxic conditions in the dark. As compared to the Calvin cycle, the energetics of triosephosphate formation from CO_2 are much more favourable ($\leqq$ 4 ATP consumed/triosephosphate formed) than the Calvin cycle (9 ATP/triosephosphate) (Diekert & Wohlfarth 1994a).

In addition, carbon monoxide dehydrogenase plays an important role in the catabolism of acetate-utilizing sulfidogens (see chapter T. Hansen) and acetoclastic methanogens (see chapter M. Blaut). Several sulfidogenic bacteria can grow with acetate plus sulfate as energy sources. Acetate is oxidized to CO_2, and the reducing equivalents are transferred to sulfate. Two pathways can be involved in acetate oxidation: in some sulfidogens a reversed CO dehydrogenase pathway is operative, in others the tricarboxylic acid cycle is used for acetate oxidation (Thauer et al. 1989) (see chapter T. Hansen).

Acetoclastic methanogens like *Methanosarcina barkeri* are able to utilize acetate as sole energy source; acetate is fermented to CO_2 plus CH_4 (Ferry 1992) (see also chapter M. Blaut). Acetate activation to acetyl-CoA is followed by subsequent cleavage of the latter component to a bound carbon monoxide and a bound methyl group. This reaction is mediated by carbon monoxide dehydrogenase. CO is then oxidized to CO_2 and the methyl group is reduced to methane.

Conclusions

Homoacetogenic bacteria are probably the most versatile anaerobes with respect to their substrate spectrum. Indeed, the 'substrate list' given above does not lay claim to completeness. Often substrates can be utilized by homoacetogens which for a long time have been considered as not easily degradable under anaerobic conditions, e.g. methyl chloride or methylarylethers. Due to their high metabolic potential and their extraordinary metabolism the homoacetogens represent an interesting, heterogenous group of microorganisms, the metabolism of which is in part still enigmatic and remains to be elucidated.

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References

Adamse AD (1980) New isolation of *Clostridium aceticum* (Wieringa). Antonie van Leeuwenhoek J. Microbiol. Serol. 46: 523–531

Andreesen JR & Ljungdahl LG (1974) Nicotinamide adenine dinucleotide phosphate dependent formate dehydrogenase from *Clostridium thermoaceticum*: Purification and properties. J. Bacteriol. 120: 6–14

Bache R & Pfennig N (1981) Selective isolation of *Acetobacterium woodii* on methoxylated aromatic acids and determination of growth yields. Arch. Microbiol. 130: 255–261

Bak F, Finster K & Rothfuß F (1992) Formation of dimethylsulfide and methanethiol from methoxylated aromatic compounds and inorganic sulfide by newly isolated anaerobic bacteria. Arch. Microbiol. 157: 529–534

Balch WE, Schoberth S, Tanner RS & Wolfe RS (1977) *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. Int. J. Sys. Bacteriol. 27: 355–361

Barker HA & Kamen MD (1945) Carbon dioxide utilization in the synthesis of acetic acid by *Clostridium thermoaceticum*. Proc. Natl. Acad. Sci. USA 31: 219–225

Bastian NR, Diekert G, Niederhoffer EC, Teo BK, Walsh CT & Orme-Johnson WH (1988) Nickel and iron EXAFS of carbon monoxide dehydrogenase from *Clostridium thermoaceticum* strain DSM. J. Am. Chem. Soc. 110: 5581–5582

Becher B, Müller V & Gottschalk G (1992) The methyltetrahydromethanopterin:coenzyme M methyltransferase of *Methanosarcina* strain Göl is a primary sodium pump. FEMS Microbiol. Lett. 91: 239–244

Berman MH & Frazer AC (1992) Importance of tetrahydrofolate and ATP in the anaerobic O-demethylation reaction for phenylmethylethers. Appl. Environ. Microbiol. 58: 925–931

Braun M, Mayer F & Gottschalk G (1981) *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. Arch. Microbiol. 128: 288–293

Breznak JA (1992) The genus *Sporomusa*. In: Balows A, Trüper HG, Dworkin M, Harder W & Schleifer KH (Eds) The prokaryotes, vol 1 (pp 517–533). Springer-Verlag, New York

Clark JE & Ljungdahl LG (1984) Purification and properties of 5,10-methylenetetrahydrofolate reductase, an iron-sulfur flavoprotein from *Clostridium formicoaceticum*. J. Biol. Chem. 259: 10845–10849

Daniel SL & Drake HL (1993) Oxalate- and glyoxylate-dependent growth and acetogenesis by *Clostridium thermoaceticum*. Appl. Environ. Microbiol. 59: 3062–3069

Daniel SL, Keith ES, Yang H, Lin YS & Drake HL (1991) Utilization of methoxylated aromatic compounds by the acetogen *Clostridium thermoaceticum:* expression and specificity of the CO-dependent O-demethylating activity. Biochem. Biophys. Res. Comm. 180: 416–422

Daniel SL, Wu Z & Drake HL (1988) Growth of thermophilic acetogenic bacteria on methoxylated aromatic acids. FEMS Microbiol. Lett. 52: 25–28

Diekert G (1992) The acetogenic bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W & Schleifer KH (Eds) The prokaryotes, vol 1 (pp 517–533). Springer-Verlag, New York

Diekert GB, Graf EG & Thauer RK (1979) Nickel requirement for carbon monoxide dehydrogenase formation in *Clostridium pasteurianum*. Arch. Microbiol. 122: 117–120

Diekert G, Hansch M & Conrad R (1984) Acetate synthesis from 2 CO_2 in acetogenic bacteria: is carbon monoxide an intermediate? Arch. Microbiol. 138: 224–228

Diekert G & Ritter M (1983) Purification of the nickel protein carbon monoxide dehydrogenase of *Clostridium thermoaceticum*. FEBS Lett. 151: 41–44

Diekert G, Schrader E & Harder W (1986) Energetics of CO formation and CO oxidation in cell suspensions of *Acetobacterium woodii*. Arch. Microbiol. 144: 386–392

Diekert G & Thauer RK (1978) Carbon monoxide oxidation by *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. J. Bacteriol. 136: 597–606

Diekert G & Thauer RK (1980) The effect of nickel on carbon monoxide dehydrogenase formation in *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. FEMS Microbiol. Lett. 7: 187–189

Diekert G & Wohlfarth G (1994a) Energetics of acetogenesis from C_1-units. In: Drake HL (Ed) Acetogenesis (in press). Chapman and Hall, New York

Diekert G & Wohlfarth G (1994b) Kohlenmonoxid im Stoffwechsel strikt anaerober Bakterien. Bioengineering, 1/94: 25–32

Dorn M, Andreesen JR & Gottschalk G (1978) Fermentation of fumerate and L-malate by *Clostridium formicoaceticum*. J. Bacteriol. 133: 26–32

Drake HL, Hu SI & Wood HG (1981) Purification of five components from *Clostridium thermoaceticum* which catalyze synthesis of acetate from pyruvate and methyltetrahydrofolate. J. Biol. Chem. 256: 11137–11144

Eichler B & Schink B (1984) Oxidation of primary aliphatic alcohols by *Acetobacterium carbinolicum* sp. nov., a homoacetogenic anaerobe. Arch. Microbiol. 140: 147–152

Elliott JI & Ljungdahl LG (1982) Isolation and characterization of an Fe_8-S_8 ferredoxin (ferredoxin II) from *Clostridium thermoaceticum*. J. Bacteriol. 151: 328–333

Ferry JG (1992) Methane from acetate. J. Bacteriol. 174: 5489–5495

Fischer F, Lieske R & Winzer K (1932) Biologische Gasreaktionen, II. Mitteilung: über die Bildung von Essigsäure bei der biologischen Umsetzung von Kohlenoxyd und Kohlensäure mit Wasserstoff zu Methan. Biochem. Z. 245: 2–12

Fontaine FE, Peterson WH, McCoy E & Johnson MJ (1942) A new type of glucose fermentation by *Clostridium thermoaceticum* n. sp. J. Bacteriol. 43: 701–715

Fuchs G (1986) CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol. Rev. 39: 181–213

Geerligs G, Aldrich HC, Harder W & Diekert G (1987) Isolation and characterization of a carbon monoxide utilizing strain of the acetogen *Peptostreptococcus productus*. Arch. Microbiol. 148: 305–313

Geerligs G, Schönheit P & Diekert G (1989) Sodium dependent acetate formation from CO_2 in *Peptostreptococcus productus* (strain Marburg). FEMS Microbiol. Lett. 57: 253–258

Genthner BRS & Bryant MP (1982) Growth of *Eubacterium limosum* with carbon monoxide as the energy source. Appl. Environ. Microbiol. 43: 70–74

Genthner BRS, Davis CL & Bryant MP (1981) Features of rumen and sewage sludge strains of *Eubacterium limosum*, a methanol- and H_2-CO_2-utilizing species. Appl. Environ. Microbiol. 42: 12–19

Gottwald M, Andreesen JR, LeGall J & Ljungdahl LG (1975) Presence of cytochrome and menaquinone in *Clostridium formicoaceticum* and *Clostridium thermoaceticum*. J. Bacteriol. 122: 325–328

Grethlein AJ, Worden RM, Jain MK & Datta R (1991) Evidence for production of n-butanol from carbon monoxide by *Butyribacterium methylotrophicum*. J. Ferm. Bioeng. 72: 58–60

Hamlett NV & Blaylock BA (1969) Synthesis of acetate from methanol. Bacteriol. Proc.: 207

Hansen B, Bokranz M, Schönheit P & Kröger A (1988) ATP formation coupled to caffeate reduction by H_2 in *Acetobacterium woodii* NZva16. Arch. Microbiol. 155: 447–451

Heise R, Müller V & Gottschalk G (1989) Sodium dependence of acetate formation by the acetogenic bacterium *Acetobacterium woodii*. J. Bacteriol. 171: 5473–5478

Heise R, Müller V & Gottschalk G (1992) Presence of a sodium-translocating ATPase in membrane vesicles of the homoacetogenic bacterium *Acetobacterium woodii*. Eur. J. Biochem. 206: 553–557

Heise R, Müller V & Gottschalk G (1993) Acetogenesis and ATP synthesis in *Acetobacterium woodii* are coupled via a transmembrane primary sodium ion gradient. FEMS Microbiol. Lett. 112: 261–268

Heyer H, Stal L & Krumbein WE (1989) Simultaneous heterolactic and acetate fermentation in the marine cyanobacterium *Oscillatoria limosa* incubated anaerobically in the dark. Arch. Microbiol. 151: 558–564

Hsu T, Daniel SL, Lux MF & Drake HL (1990) Biotransformations of carboxylated aromatic compounds by the acetogen *Clostridium thermoaceticum*: generation of growth-supportive CO_2 equivalents under CO_2-limited conditions. J. Bacteriol. 172: 212–217

Hugenholtz J, Ivey DM & Ljungdahl LG (1987) Carbon monoxide-driven electron transport in *Clostridium thermoautotrophicum* membranes. J. Bacteriol. 169: 5845–5847

Hugenholtz J & Ljungdahl LG (1990) Metabolism and energy generation in homoacetogenic clostridia. FEMS Microbiol. Rev. 87: 383–390

Kamlage B & Blaut M (1993) Isolation of a cytochrome-deficient mutant strain of *Sporomusa sphaeroides* not capable of oxidizing methyl groups. J. Bacteriol. 175: 3043–3050

Kamlage B, Boelter A & Blaut M (1993) Spectroscopic and potentiometric characterization of cytochromes in two *Sporomusa* species and their expression during growth on selected substrates. Arch. Microbiol. 159: 189–196

Kreft JU & Schink B (1993) Demethylation and degradation of phenylmethylethers by the sulfide-methylating homoacetogenic bacterium strain TMBS 4. Arch. Microbiol. 159: 308–315

Kröger A, Geisler V, Lemma E, Theis F & Lenger R (1992) Bacterial fumarate respiration. Arch. Microbiol. 158: 311–314

Kumar M, Lu WP, Liu L & Ragsdale SW (1993) Kinetic evidence that carbon monoxide dehydrogenase catalyzes the oxidation of carbon monoxide and the synthesis of acetyl-CoA at separate metal centers. J. Am. Chem. Soc. 115: 11646–11647

Lee MJ & Zinder SH (1988a) Isolation and characterization of a thermophilic bacterium which oxidizes acetate in syntrophic association with a methanogen and which grows acetogenically on H_2-CO_2. Appl. Environ. Microbiol. 54: 124–129

Lee MJ & Zinder SH (1988b) Carbon monoxide pathway enzyme activities in a thermophilic anaerobic bacterium grown acetogenically and in a syntrophic acetate-oxidizing coculture. Arch. Microbiol. 150: 513–518

Ljungdahl LG (1986) The autotrophic pathway of acetate synthesis in acetogenic bacteria. Ann. Rev. Microbiol. 40: 415–450

Ljungdahl LG, Brewer JM, Neece SH & Fairwell T (1970) Purification, stability, and composition of formyltetrahydrofolate synthetase from *Clostridium thermoaceticum*. J. Biol. Chem. 245: 4791–4797

Ljungdahl LG, Irion E & Wood HG (1966) Role of corrinoids in the total synthesis of acetate from CO_2 by *Clostridium thermoaceticum*. Fed. Proc. Am. Soc. Exp. Biol. 25: 1642–1648

Ljungdahl LG & Wood HG (1969) Total synthesis of acetate from CO_2 by heterotrophic bacteria. Annu. Rev. Microbiol. 23: 515–538

Ljungdahl LG & Wood HG (1982) Acetate biosynthesis. In: Dolphin D (Ed) Vitamin B_{12} (pp 165–202). Wiley, New York

Lorowitz WH & Bryant MP (1984) *Peptostreptococcus productus* strain that grows rapidly with CO as the energy source. Appl. Environ. Microbiol. 47: 961–964

Ma K, Siemon S & Diekert G (1987) Carbon monoxide metabolism in cell suspensions of *Peptostreptococcus productus* strain Marburg. FEMS Microbiol. Lett. 43: 367–371

Margheri MC & Allotta G (1993) Homoacetic fermentation in the cyanobacterium *Nostoc* sp. strain Cc from *Cycas circinalis*. FEMS Microbiol. Lett. 111: 213–218

Matthies C, Freiberger A & Drake HL (1993) Fumarate dissimilation and differential reductant flow by *Clostridium formicoaceticum* and *Clostridium aceticum*. Arch. Microbiol. 160: 273–278

Meßmer M, Wohlfarth G & Diekert G (1993) Methyl chloride metabolism of the strictly anaerobic, methyl chloride-utilizing homoacetogen strain MC. Arch. Microbiol. 160: 383–387

Möller B, Ossmer R, Howard BH, Gottschalk G & Hippe H (1984) *Sporomusa*, a new genus of gram-negative anaerobic bacteria including *Sporomusa sphaeroides* spec. nov. and *Sporomusa ovata* spec. nov. Arch. Microbiol. 139: 388–396

Moore MR, O'Brien WE & Ljungdahl LG (1974) Purification and characterization of nicotinamide adenine dinucleotide-dependent methylenetetrahydrofolate dehydrogenase from *Clostridium formicoaceticum*. J. Biol. Chem. 249: 5250–5253

O'Brien WE, Brewer JM & Ljungdahl LG (1973) Purification and characterization of thermostable, 5,10-methylenetetrahydrofolate dehydrogenase from *Clostridium thermoaceticum*. J. Biol. Chem. 248: 403–408

Plugge CM, Dijkema C & Stams AJM (1993) Acetyl-CoA cleavage pathway in a syntrophic propionate oxidizing bacterium growing on fumarate in the absence of methanogens. FEMS Microbiol. Lett. 110: 71–76

Poston JM, Kuratomi K & Stadtman ER (1964) Methyl-vitamin B_{12} as a source of methyl groups for the synthesis of acetate by cell-free extracts of *Clostridium thermoaceticum*. Ann. N.Y. Acad. Sci. 112: 804–806

Ragsdale SW (1991) Enzymology of the acetyl-CoA pathway of CO_2 fixation. Crit. Rev. Biochem. Mol. Biol. 26: 261–300

Ragsdale SW, Clark JE, Ljungdahl LG, Lundie LL & Drake HL (1983a) Properties of purified carbon monoxide dehydrogenase from *Clostridium thermoaceticum*, a nickel, iron-sulfur protein. J. Biol. Chem. 258: 2364–2369

Ragsdale SW, Lindahl PA & Münck E (1987) Mössbauer, EPR, and optical studies of the corrinoid/iron-sulfur protein involved in the synthesis of acetyl coenzyme A by *Clostridium thermoaceticum*. J. Biol. Chem 262: 14289–14297

Ragsdale SW, Ljungdahl LG & der Vartanian DV (1983b) Isolation of carbon monoxide dehydrogenase from *Acetobacterium woodii* and comparison of its properties with those of the *Clostridium thermoaceticum* enzyme. J. Bacteriol. 155: 1224–1237

Ragsdale SW, Wood HG & Antholine WE (1985) Evidence that an iron-nickel-carbon complex is formed by reaction of CO with CO dehydrogenase from *Clostridium thermoaceticum*. Proc. Natl. Acad. Sci. USA 82: 6811–6814

Reubelt U, Wohlfarth G, Schmid R & Diekert G (1991) Purification and characterization of ferredoxin from *Peptostreptococcus productus* (strain Marburg). Arch. Microbiol. 156: 422–426

Roberts JR, Lu WP & Ragsdale SW (1992) Acetyl-coenzyme-A synthesis from methyl-tetrahydrofolate, CO, and coenzyme-A by enzymes purified from *Clostridium thermoaceticum* – attainment of *in vivo* rates and identification of rate-limiting steps. J. Bacteriol. 174: 4667–4676

Schramm E & Schink B (1991) Ether-cleaving enzyme and diol dehydratase involved in anaerobic polyethylene glycol degradation by a new *Acetobacterium* sp. Biodeg. 2: 71–79

Schulman M, Ghambeer RK, Ljungdahl LG & Wood HG (1973) Total synthesis of acetate from CO_2. VII. Evidence with *Clostridium thermoaceticum* that the carboxyl group of acetate is derived from the carboxyl of pyruvate by transcarboxylation and not by fixation of CO_2. J. Biol. Chem. 248: 6255–6261

Schuppert B & Schink B (1990) Fermentation of methoxyacetate to glycolate and acetate by newly isolated strains of *Acetobacterium* sp. Arch. Microbiol. 153: 200–204

Seifritz C, Daniel SL, Gößner A & Drake HL (1993) Nitrate as a preferred electron sink for the acetogen *Clostridium thermoaceticum*. J. Bacteriol. 175: 8008–8013

Shin W, Anderson ME & Lindahl PA (1993) Heterogenous nickel environments in carbon monoxide dehydrogenase from *Clostridium thermoaceticum*. J. Am. Chem. Soc. 115: 5522–5526

Stupperich E & Konle R (1993) Corrinoid-dependent methyl transfer reactions are involved in methanol and 3,4-dimethoxybenzoate metabolism by *Sporomusa ovata*. Appl. Environ. Microbiol. 59: 3110–3116

Tananka K & Pfennig N (1988) Fermentation of 2-methoxyethanol by *Acetobacterium malicum* sp. nov. and *Pelobacter venetianus*. Arch. Microbiol. 149: 181–187

Tanner RS, Miller LM & Yang D (1993) *Clostridium ljungdahlii* sp. nov., an acetogenic species in clostridial rRNA homology group I. Int. J. Sys. Bacteriol. 43: 232–236

Thauer RK, Jungermann K & Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41: 100–180

Thauer RK, Möller-Zinkhan D & Spormann AM (1989) Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. Ann. Rev. Microbiol. 43: 43–67

Traunecker J, Preuß A & Diekert G (1991) Isolation and characterization of a methyl chloride utilizing, strictly anaerobic bacterium. Arch. Microbiol. 156: 416–421

Tschech A & Pfennig N (1984) Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. Arch. Microbiol. 137: 163–167

Van der Meijden P, van der Drift C & Vogels GD (1984) Methanol conversion in *Eubacterium limosum*. Arch. Microbiol. 138: 360–364

Wagener S & Schink B (1988) Fermentative degradation of nonionic surfactants and polyethylene glycol by enrichment cultures and by pure cultures of homoacetogenic and propionate-forming bacteria. Appl. Environ. Microbiol. 54: 561–565

Whitman WB, Bowen TL & Boone DR (1992) The methanogenic bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W & Schleifer KH (Eds) The prokaryotes, vol 1 (pp 719–767). Springer-Verlag, New York

Wieringa KT (1936) Over het verdwijnen van waterstof en koolzuur onder anaerobe voorwaarden; voortzetting van een onderzoek van wijlen Prof. Dr. Ir. N.L. Söhngen. Antonie van Leeuwenhoek J. Microbiol. Serol. 3: 263–273

Winters DK & Ljungdahl LG (1989) PQQ-dependent methanol dehydrogenase from *Clostridium thermoautotrophicum*. In: Jongejan JA & Duine JA (Eds) PQQ and quinoproteins (pp 35–39). Kluwer Academic Publishers, Dordrecht Boston London

Wohlfarth G & Diekert G (1991) Thermodynamics of methylenetetrahydrofolate reduction to methyltetrahydrofolate and its implications for the energy metabolism of homoacetogenic bacteria. Arch. Microbiol. 155: 378–381

Wohlfarth G, Geerligs G & Diekert G (1990) Purification and properties of a NADH dependent 5,10-methylenetetrahydrofolate reductase from *Peptostreptococcus productus*. Eur. J. Biochem. 192: 411–417

Wood HG (1952) A study of carbon dioxide fixation by mass determination of the types of C^{13}-acetate. J. Biol. Chem. 194: 905–931

Wood HG (1991) Life with CO or CO_2 and H_2 as a source of carbon and energy. FASEB J. 5: 156–163

Yang H & Drake HL (1990) Differential effects of sodium on hydrogen- and glucose-dependent growth of the acetogenic bacterium *Acetogenium kivui*. Appl. Environ. Microbiol. 56: 81–86

Zeikus JG, Lynd LH, Thompson TE, Krzycki JA, Weimer PJ & Hegge PW (1980) Isolation and characterization of a new, methylotrophic, acidogenic anaerobe, the Marburg strain. Curr. Microbiol. 3: 381–386