The Wolfe cycle comes full circle

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In 1988, Rouvière and Wolfe (1) suggested that methane formation from H_2 and CO_2 by methanogenic archaea could be a cyclical process. Indirect evidence indicated that the first step, the reduction of CO_2 to formylmethanofuran, was somehow coupled to the last step, the reduction of the heterodisulfide (CoM-S-S-CoB) to coenzyme M (CoM-SH) and coenzyme B (CoB-SH). Over 2 decades passed until the coupling mechanism was unraveled in 2011: Via flavin-based electron bifurcation, the reduction of CoM-S-S-CoB with H_2 provides the reduced ferredoxin (Fig. 1h) required for CO_2 reduction to formylmethanofuran (2) (Fig. 1a). However, one question still remained unanswered: How are the intermediates replenished that are removed for the biosynthesis of cell components from CO_2 (orange arrows in Fig. 1)? This anaplerotic (replenishing) reaction has recently been identified by Lie et al. (3) as the sodium motive force-driven reduction of ferredoxin with H_2 catalyzed by the energy-converting hydrogenase EhaA-T (green arrow in Fig. 1).

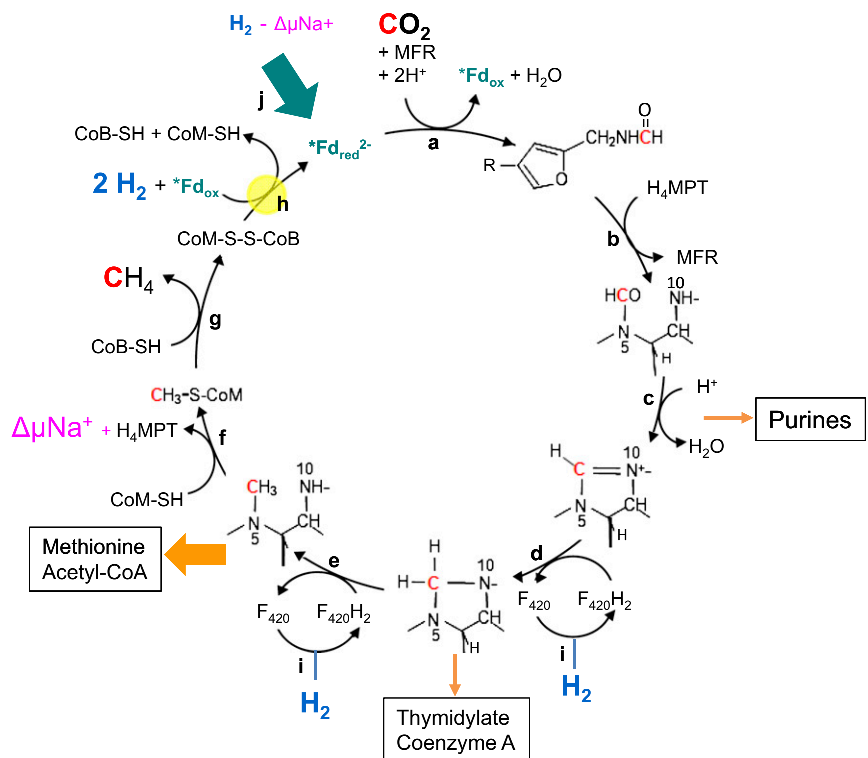

Fig. 1. Wolfe cycle of CO_2 reduction to methane with 4 H_2 in hydrogenotrophic methanogenic archaea. Orange arrows indicate biosynthetic reactions that remove intermediates (thickness of the arrows reflects the quantitative importance), the green arrow illustrates the anaplerotic reaction catalyzed by EhaA-T, and yellow highlighting represents the electron-bifurcating reaction. Reactions f and j are catalyzed by membrane-associated enzyme complexes. All other reactions are catalyzed by cytoplasmic enzymes. F_{420}, coenzyme F_{420}; *Fd, specific ferredoxin; H_4MPT, tetrahydromethanopterin; MFR, methanofuran; ΔμNa⁺, electrochemical sodium ion potential. Enzymes: a, formylmethanofuran dehydrogenase; b, formylmethanofuran/H_4MPT formyltransferase; c, methenyl-H_4MPT cyclohydrolase; d, methylene-H_4MPT dehydrogenase; e, methylene-H_4MPT reductase; f, methyl-H_4MPT/coenzyme M methyltransferase; g, methyl-coenzyme M reductase; h, electron-bifurcating hydrogenase–heterodisulfide reductase complex; i, F_{420}-reducing hydrogenase; j, energy-converting hydrogenase catalyzing the sodium motive force-driven reduction of ferredoxin with H_2. With *Methanothermobacter marburgensis*, it has been shown that one of the two C_1 carbons in purines is derived from formate (C-2) and the other is derived from formyl-H_4MPT/methenyl-H_4MPT (C-8) (25).

Biological methane formation from H_2 and CO_2 ($\Delta G^{\circ\prime}$ = −131 kJ/mol) is not only a quantitatively important process but possibly one of the oldest (4). It involves such coenzymes as methanofuran, tetrahydromethanopterin (H_4MPT), and CoM-SH as C_1-unit carriers. These coenzymes were first thought to be unique to anaerobic methanogenic archaea (5) but were later found also in methanotrophic bacteria (6) and in some other microorganisms (7, 8). CO_2 reduction to methane begins with its reduction to formylmethanofuran with reduced ferredoxin (Fd_{red}^{2-}) (9–11), which is regenerated by reduction of oxidized ferredoxin (Fd_{ox}) with H_2 (12). The redox potential of the Fd_{ox}/Fd_{red}^{2-} couple of −500 mV is almost 200 mV more negative than that of the $2H^+/H_2$ couple at a pH of 7 and the H_2 partial pressure of 10 Pa prevailing in the anaerobic habitats of hydrogenotrophic methanogens (11). The endergonic reduction of ferredoxin with H_2 must therefore somehow be coupled to one of the three downstream exergonic reactions.

The first exergonic reaction is the transfer of the methyl group from methyl-H_4MPT to CoM-SH ($\Delta G^{\circ\prime}$= −30 kJ/mol), which is catalyzed by the membrane-associated and energy-conserving enzyme complex MtrA-H (13) (Fig. 1f). The reaction is associated with the build-up of an electrochemical sodium ion potential, which, in turn, drives both ATP synthesis via the membrane-associated A_oA_1-ATP synthase (not shown) and ferredoxin reduction with H_2 via the membrane-associated and energy-converting hydrogenase complexes EhaA-T (Fig. 1j) and EhbA-Q (not shown) (11).

The second exergonic reaction is the reduction of methyl-coenzyme M with CoB-SH to methane and the heterodisulfide CoM-S-S-CoB ($\Delta G^{\circ\prime}$ = −30 kJ/mol) catalyzed by cytoplasmic methyl-coenzyme M reductase (Fig. 1g). As far as known, this reaction is not coupled with ferredoxin reduction or energy conservation (14, 15).

The third exergonic reaction is the reduction of CoM-S-S-CoB with H_2 ($\Delta G^{\circ\prime}$ = −55 kJ/mol) catalyzed by a cytoplasmic hydrogenase–heterodisulfide reductase complex (Fig. 1h), which couples this reaction with the endergonic reduction of ferredoxin with H_2 ($\Delta G^{\circ\prime}$ = +16 kJ/mol) and renders the methanogenic pathway cyclical (2). Coupling is via the newly discovered mechanism of flavin-based electron bifurcation (16).

Using elegant genetic experiments, Lie et al. (3) show that when the genes for six of the seven chromosomally encoded

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hydrogenases of *Methanococcus maripaludis* are deleted, this model organism can still grow on formate, but only when H_2 is also present. Only the energy-converting hydrogenase EhaA-T appears to be essential. During growth on formate, the hydrogenase–heterodisulfide reductase complex (Fig. 1h) is substituted by an electron-bifurcating formate dehydrogenase–heterodisulfide reductase complex and the F_{420}-reducing hydrogenase (Fig. 1i) is substituted by F_{420}-reducing formate dehydrogenase. The H_2 is used in substoichiometric amounts (< 0.2 mol of H_2 per mol of CH_4) that approximately comprise the amount of H_2 required for autotrophic CO_2 fixation, which suggests an anaplerotic role of EhaA-T.

Why is an anaplerotic reaction so important? It is because intermediates are continuously withdrawn from the cycle for the biosynthesis of purines (DNA and RNA); thymidylate (DNA); CoA; methionine (protein and S-adenosyl-methionine); and, quantitatively most important, acetyl-CoA (Fig. 1), which is synthesized from methyl-H_4MPT, CO, and CoA in autotrophic methanogens (17). If reduced ferredoxin is not replenished, the cycle would come to a halt. The same would happen if electron bifurcation is imperfectly coupled (11). However, imperfect coupling appears not to be of quantitative importance, as evidenced by the relatively low H_2 requirement during growth on formate of the *M. maripaludis* mutant lacking the six hydrogenases. This finding is another important result of the work of Lie et al. (3).

The elucidation of the cycle all began in 1977 with an experiment by Gunsalus and Wolfe (18), which showed that in cell extracts of *Methanobacterium thermoautotrophicum* strain ΔH (now *Methanothermobacter thermoautotrophicus*), the reduction of CO_2 with H_2 to methane proceeds only in the presence of catalytical amounts of methyl-coenzyme M. Later, it was found that methyl-coenzyme M could be substituted by catalytical amounts of CoM-S-S-CoB (19). This finding was reminiscent of the observation of Hans Adolf Krebs (20) that in liver slices, the formation of urea from ammonia and CO_2 is stimulated by catalytical amounts of ornithine, which led to the discovery of the first metabolic cycle, the urea cycle. This was followed by Krebs' elucidation of the tricarboxylic acid cycle for acetate oxidation. Later, Hans L. Kornberg (21) showed that anaplerotic reactions were necessary to replenish the intermediates of the Krebs cycle used for biosynthesis. With the anaplerotic reaction found by Lie et al. (3), the cycle of CO_2 reduction to methane is finally complete. This methanogenic cycle is now referred to as the Wolfe cycle, in honor of Ralph S. Wolfe (22), who has been the motor driving its elucidation.

One can argue that the Wolfe cycle is not a cycle comparable to the Krebs cycle because the first and last steps are coupled via the electron carrier ferredoxin rather than by a carbon compound. Also, the Embden–Meyerhof pathway would be a cycle if formulated with ATP as an intermediate connecting the first step and the last step, namely, the hexokinase reaction and the pyruvate kinase reaction. However, the Wolfe cycle differs from the Embden–Meyerhof pathway in that the ferredoxin involved appears to be used mainly in the cycle, whereas the ATP of the Embden–Meyerhof pathway is also used elsewhere. Evidence for a specific ferredoxin comes from the finding that the energy-converting hydrogenase EhbA-Q, which catalyzes the sodium motive force-driven reduction of ferredoxin with H_2, has a purely anabolic function and cannot substitute for the EhaA-T complex catalyzing the same reaction. This can be explained by assuming that the two hydrogenases prefer to use different ferredoxins. Indeed, in the genome of all methanogens analyzed, several genes for ferredoxins with quite different predicted structures are encoded (23). An alternative explanation is that ferredoxin pools are compartmentalized. The finding that the hydrogenase–heterodisulfide reductase complex (Fig. 1h) and the formylmethanofuran dehydrogenase complex (Fig. 1a) form super complexes points in this direction (24).

How widespread is the Wolfe cycle? Biochemical and genomic information indicates that with modifications, the cycle operates in all members of the Methanobacteriales, Methanopyrales, Methanococcales, Methanomicobiales, and Methanocellales that can grow on H_2/CO_2 and/or formate. Only the relatively few members of the Methanosarcinales that grow on H_2 and CO_2 are exceptions, although these members appear to contain all the genes required for the operation of the cycle. In *Methanosarcina barkeri*, for example, the reduction of CoM-S-S-CoB is catalyzed by two membrane-associated enzyme complexes and is associated with the build-up of an electrochemical proton potential, which, in turn, drives the reduction of the ferredoxin required for CO_2 reduction to formylmethanofuran via an energy-converting hydrogenase (11). In *M. barkeri* during growth on H_2 and CO_2, the function of the energy-converting hydrogenase is therefore catabolic rather than anaplerotic.

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