Chapter 2
Microbial CO Metabolism

CO metabolism begins with a reaction that can be considered a thermodynamically favorable disproportionation, resulting in CO_2 and a pair of reducing equivalents, or molecular hydrogen as products:

$$CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$$

The enzyme catalyzing this reaction is carbon monoxide dehydrogenase (CODH). This enzyme functions to either oxidize CO, synthesize acetyl-CoA, or cleave acetyl-CoA in a variety of energy-yielding pathways (Hausinger 1993). It contains iron (non-heme) and either molybdenum (in aerobes) or nickel (in anaerobes) in the active site (Ragsdale 2004). In aerobic carboxydotrophs, the CODH is monofunctional, which consist of a dimer of heterotrimers containing FAD and molybdopterin cytosine dinucleotide cofactors (Dobbek et al. 2002). The high-resolution crystal structures of the MoFeS CODHs from *Oligotropha carboxidovorans* (Gremer et al. 2000) or *Hydrogenophaga pseudoflava* (Hanzelmann et al. 2000) show a dimer of two heterotrimers in a $(LMS)_2$ subunit structure. Each heterotrimer is composed of a molybdenum protein (L subunit), a flavoprotein (M subunit), and an iron–sulfur protein (S subunit). The molybdoprotein carries the active site, which contains a 1:1 molar complex molybdopterin cytosine dinucleotide and a molybdenum atom. The iron–sulfur protein contains the type I and type II [2Fe-2S] centers. The flavoprotein contains the flavin adenine dinucleotide (FAD) cofactor and shows a new flavin-binding type (Gremer et al. 2000). The genes encoding the aerobic CODH are denoted by *cox* (carbon monoxide oxidase genes) (Hugendieck and Meyer 1992). Two forms of aerobic CODH have been identified so far: form I and form II. Form I has been specifically characterized for its ability to oxidize CO. Form II, which is phylogenetically close it, but distinct from form I, is a putative CODH, the true function of which remains uncertain. In contrast to form II CODH, the active site of form I CODH contains a unique catalytically essential loop of four amino acids, cysteine, serine, phenylalanine, and arginine, and a copper atom linked to the active site cysteine sulfur and to the molybdenum atom. In these bacteria, the

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reducing equivalents resulting from CO oxidation are funneled through a CO-insensitive respiratory chain via ubiquinone and cytochromes ultimately resulting in oxygen reduction (Frunzke and Meyer 1990).

Aerobic CO oxidation can be viewed analogous to aerobic H_2 oxidation carried out by knallgas bacteria (Schlegel 1966); however, H_2 is not an intermediate in CO-dependent O_2 reduction (Meyer and Schlegel 1983). In some aerobic carboxydotrophs, energy conserved from CO metabolism can be used to fix CO_2 to biomass. This process typically involves the Calvin–Benson–Bassham (CBB) cycle, which is based on the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Ragsdale 2004). Other aerobic carboxydotrophs seem unable to fix CO_2 by the CBB cycle or other mechanisms. In these organisms, CO can provide a supplemental energy source without contributing directly to biomass.

Anaerobic carboxydotrophs have CODHs that differ distinctly from that of aerobic carboxydotrophs, in part because it contains nickel instead of molybdenum as a metal cofactor. The NiFeS CODHs are either monofunctional or bifunctional (Ferry 1995). They form complex with acetyl coenzyme A (acetyl-CoA) synthase (ACS). The monofunctional CODH from phototrophic bacterium *Rhodospirillum rubrum* (Bonam and Ludden 1987) is inducible in the dark under anaerobic conditions in the presence of CO, shows a micromolar K_m for CO (Bonam and Ludden 1987), and contains a proposed nickel–iron–sulfur cluster (cluster C) (Heo et al. 1999) and a conventional [4Fe–4S] cluster (cluster B) (Hu et al. 1996). Radiolabeling studies suggested a catalytically essential non-substrate CO ligand (CO_L) to the Fe atom in putative [Fe–Ni] center cluster C (Heo et al. 2000). Acetogens and methanogens employ the bifunctional CODH–ACS (Ferry 1995). The enzymes are tetramers of two different subunits or pentamers of five different subunits. The subunits harboring the CODH activity contain cluster C and cluster B (Ferry 1995), which is similar to the function of *R. rubrum* CODH. Phototrophic anaerobic carboxydotrophs grow by converting CO to H_2, a process initiating with CODH that contains nickel and iron–sulfur centers (Drennan 1991). Acetogenic carboxydotrophs employ nickel/iron–sulfur CODH to synthesize acetyl-CoA from a methyl group, CO, and CoA (Ragsdale and Kumar 1996). A similar enzyme is responsible for the cleavage of acetyl-CoA by anaerobic *Archaea* that obtain energy by fermenting acetate to CH_4 and CO_2. Sulfate-reducing carboxydotrophs from *bacteria* and *archaea* also utilize CODH to cleave acetyl-CoA yielding methyl and carbonyl groups. These microbes obtain energy for growth via a respiratory pathway in which the methyl and carbonyl groups are oxidized to CO_2, and the sulfate is reduced to sulfide (Ferry 1995). CO is considered an excellent source of energy since the redox potential ($E^{o\prime}$) of the CO_2/CO couple is very low (−524 to 558 mV) (Grahame and DeMoll 1995). However, relatively few anaerobic microbes capable of utilizing CO as their sole source of energy have been described. Known respiratory processes, which are coupled to anaerobic CO oxidation, are illustrated in Fig. 2.1. These respiratory processes include proton respiration (hydrogenogenesis) (Henstra et al. 2007b), sulfate (or sulfur) respiration (desulfurication) (Rabus et al. 2006), and carbonate respiration (acetogenesis and methanogenesis) (Drake et al. 2002).

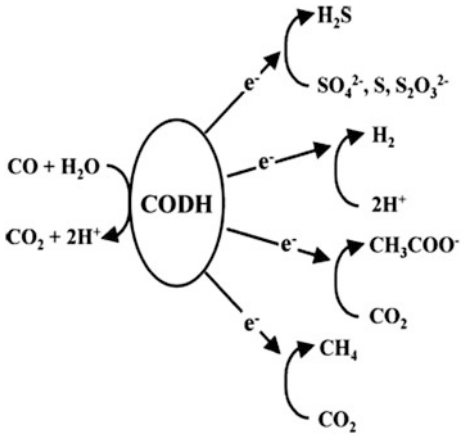

Fig. 2.1 Scheme of anaerobic respirations, which can be coupled to CO oxidation. CODH carbon monoxide dehydrogenase; $-e^{-}\rightarrow$ electron transport chain, some components of which are unknown (Oelgeschlager and Rother 2008)

Anaerobic carboxydotrophic hydrogenogenic microorganisms conserve energy by oxidation of CO to CO_2 coupled to reduction of protons to H_2. These reactions are catalyzed by CO dehydrogenase and hydrogenase, respectively. These two enzymes must conserve energy in a yet-unknown energy-conserving mechanism as they do not fit in classical substrate-level phosphorylation (SLP) and electron transfer phosphorylation (ETP) theories (Hedderich 2004). Hydrogenases catalyze the reduction of protons to H_2 or the reverse reaction according to:

$$2H^{+} + 2e^{-} \leftrightarrow H_2$$

The physiological function of hydrogenases is generally restricted to one of these directions and is referred to as hydrogen-uptake hydrogenase or hydrogen-evolving hydrogenase. Recently, the classification and phylogeny of hydrogenases were reviewed by Vignais et al. (2001). Three classes of hydrogenases are recognized based on phylogeny and metal content (also transition-metal content or H_2-activating site content). The first and largest class is formed by the [NiFe]-hydrogenases. The second class, [FeFe]-hydrogenases only, contains Fe in their active site, while the third class is formed by hydrogenases that until recently were named "metal-free" hydrogenases (Berkessel and Thauer 1995). The latter class was discovered in methanogens, where they catalyze the reduction of F_{420} with H_2 in complex with methylenetetrahydromethanopterin dehydrogenase under nickel-deprived conditions (Zirngibl et al. 1992; Afting et al. 1998, 2000). Now they are referred to as iron–sulfur cluster-free hydrogenases, since the presence of a novel light-sensitive iron-coordinating cluster in this type of hydrogenases was found (Buurman et al. 2000; Lyon et al. 2004; Shima et al. 2004).

Awareness that a unique subclass of [NiFe]-hydrogenases exists has grown in the past decade. These hydrogenases were indicated as energy-converting hydrogenases (ECH), after their capacity to couple proton translocation to the

reduction of protons or oxidation of molecular H_2, and were recently reviewed by Hedderich et al. (2004). A limited sequence similarity and a deviating enzyme topology mark the main differences between ECH and other [NiFe]-hydrogenases. These ECH are membrane-bound enzyme complexes and play a key role in energy generation in the carboxydotrophic hydrogenogenic metabolism (Hedderich et al. 2004). The ECH couples the oxidation of H_2 or reduction of protons to translocation of protons over the cytoplasmic membrane. The electrochemical gradient of protons over the membrane is generally referred to as proton motive force (pmf) and is the driving force for ATP synthesis. Translocation of protons by ECH may generate the pmf depending on its direction. Reduction of protons to form H_2 is coupled to the generation of a pmf, which in turn drives ATP synthesis. The number of suitable electron donors for reduction of the protons is limited by the relatively low electrode potential of the H^+/H_2 couple ($E^{\circ\prime}$ −414 mV). The CO_2/CO couple ($E^{\circ\prime}$ −520 mV) is sufficiently low to drive proton translocation by ECH, but also formate or reduced ferredoxin generated by pyruvate:ferredoxin oxidoreductase in fermentative metabolisms serve as electron donors for ECH (Bagramyan and Trchounian 2003; Sapra et al. 2003; Hedderich et al. 2004; Soboh et al. 2004). Reduced ferredoxin may donate electrons directly to ECH while CODH and formate dehydrogenase form a complex with ECH. In these complexes, a ferredoxin-like subunit facilitates electron transfer. In the carboxydotrophic hydrogenogenic metabolism, ECH together with CODH play an important role as described for *Carboxydothermus hydrogenoformans* and *R. rubrum* (Hedderich et al. 2004; Fox et al. 1996a,b). These organisms contain similar enzymatic systems that catalyze the conversion of CO into H_2. Genes that code for the involved enzymes are arranged in two gene clusters that share high sequence similarity between both organisms. One cluster comprises the genes for ECH, the other cluster encodes for a CODH and *CooF*. One functional CO-oxidizing H_2-evolving complex is formed with these subunits, as was shown for *C. hydrogenoformans* (Soboh et al. 2002). Coupling of proton translocation to oxidation of CO by *C. hydrogenoformans* and *R. rubrum* enables them to use CO as sole source of energy and to grow with the formation of H_2.

The possible role of CO as electron donor in anaerobic respiration has received little attention, and the number of species known to use CO in anaerobic respiration is still limited. *Moorella thermoacetica* can grow chemolithotrophically with CO as electron donor and nitrate as electron acceptor (Drake and Daniel 2004; Frostl et al. 1996). Furthermore, *Thermosinus carboxydivorans* reduces ferric iron and selenite with CO as electron donor (Sokolova et al. 2004b), and *C. hydrogenoformans* reduces fumarate and 9,10-anthraquinone-2,6-disulfonate (AQDS) with CO as electron donor (Henstra and Stams 2004). *C. hydrogenoformans reduces* nitrate, thiosulfate, sulfur, and sulfite with lactate as electron donor, but according to Henstra and Stams (2004), CO might be able to serve as electron donor as well. However, *T. carboxydivorans* and *C. hydrogenoformans* form H_2 from CO, which might be the actual electron donor for these reductions. In contrast, *Thermoterrabacterium ferrireducens* does not form hydrogen with CO, but is able to reduce

AQDS and fumarate with CO. Besides AQDS, *T. ferrireducens* may reduce Fe (III), nitrate, sulfite, thiosulfate, and sulfur with CO as well, as it does with H_2 or lactate (Henstra and Stams 2004; Slobodkin et al. 1997).

The exact range of microorganisms capable to use CO is still unclear, as CO utilization is rarely tested in growth studies. Although CO may initially inhibit growth, adaptation to CO can occur after long-term incubation or multiple transfers with increasing CO levels (Rother and Metcalf 2004; O'Brien et al. 1984). Furthermore, growth on CO may require different nutrients or concentrations (Kerby et al. 1995). The ability of CO oxidation to CO_2 seems ubiquitously present in nature and has an ancient origin, as mentioned by Ferry (1995) and Hedderich (2004).

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