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Microbial growth on carbon monoxide

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Abstract

The utilization of carbon monoxide as energy and/or carbon source by different physiological groups of bacteria is described and compared. Utilitarian CO oxidation which is coupled to the generation of energy for growth is achieved by aerobic and anaerobic eu- and archaebacteria. They belong to the physiological groups of aerobic carboxidotrophic, facultatively anaerobic phototrophic, and anaerobic acetogenic, methanogenic or sulfate-reducing bacteria. The key enzyme in CO oxidation is CO dehydrogenase which is a molybdo iron-sulfur flavoprotein in aerobic CO-oxidizing bacteria and a nickel-containing iron-sulfur protein in anaerobic ones. In carboxidotrophic and phototrophic bacteria, the CO-born CO_2 is fixed by ribulose bisphosphate carboxylase in the reductive pentose phosphate cycle. In acetogenic, methanogenic, and probably in sulfate-reducing bacteria, CODH/acetyl-CoA synthase directly incorporates CO into acetyl-CoA.

In plasmid-harbouring carboxidotrophic bacteria, CO dehydrogenase as well as enzymes involved in CO_2 fixation or hydrogen utilization are plasmid-encoded. Structural genes encoding CO dehydrogenase were cloned from carboxidotrophic, acetogenic and methanogenic bacteria. Although they are clustered in each case, they are genetically distinct.

Soil is a most important biological sink for CO in nature. While the physiological microbial groups capable of CO oxidation are well known, the type and nature of the microorganisms actually representing this sink are still enigmatic. We also tried to summarize the little information available on the nutritional and physicochemical requirements determining the sink strength. Because CO is highly toxic to respiring organisms even in low concentrations, the function of microbial activities in the global CO cycle is critical.

Introduction

Carbon monoxide (CO) is a gaseous C_1-compound highly toxic to many living beings. Nevertheless, there exist microbes which not only are able to cope with the problems CO poses, but moreover can use it for growth as a carbon and/or energy source. Although bacteria belonging to different taxonomic and physiological groups possess different systems for CO utilization, all of these systems oxidize CO to carbon dioxide (CO_2). Energy derived from this oxidation reaction can be used for the generation of a protonmotive force across the cytoplasmic membrane leading to the formation of ATP. CO-carbon is incorporated either directly into a cell metabolite (acetyl-CoA) or its oxidation product CO_2 is fixed. The scope of this review is to summarize and compare the metabolic pathways used by bacteria for growth on CO.

Microorganisms are responsible for the consumption of CO in soil, which is one of the major sinks and the only known biological sink for CO. The increasing CO production by the activities of human beings (industry, traffic) raises the question whether the capacity of the sinks is able to keep pace with the production. An increase in the atmosphere's CO content would be dangerous from a human point of view in at least two respects: i) CO is highly toxic to many living beings and ii) it contributes to global warming in the 'greenhouse effect'.

I. The substrate: properties of carbon monoxide (CO)

Carbon monoxide (CO) is a colourless, odourless, tasteless gas. Its solubility in water is 21.4 μl · ml^{-1} H_2O (at 25° C and atmospheric pressure) and hence comparable to that of oxygen (28.3 μl O_2 · ml^{-1} H_2O), but much lower than that of carbon dioxide (759 μl CO_2 · ml^{-1} H_2O).

The CO molecule is isosteric and isoelectronic to cyanide (CN^-) and dinitrogen (N_2), and therefore many enzymes susceptible to inhibition by CN^-, e.g. heme-containing enzymes, are also inhibited by CO. The CO-utilizing systems which are discussed below are all inhibited more or less by CN^-. An exception is *Streptomyces thermoautotrophicus*, which grows on CO in the presence of CN^-. Classical nitrogenases are inhibited by CO and use CN^- as a substrate. Again, *S. thermoautotrophicus* is unusual as its N_2-fixing system is not inhibited by the presence of CO (Gadkari et al. 1992).

As a result of its special electron configuration, CO acts as a donor-ligand towards transition metals forming dπ–pπ bonds. Its high affinity for the metal ions present in components of respiratory chains, e.g. terminal cytochrome oxidase, makes it extremely toxic to aerobic organisms (Chance et al. 1970). In addition, because of its high affinity, CO binds competitively instead of oxygen to hemoglobin and myoglobin, resulting in an oxygen limitation to the respiring organisms with lethal effect (Otis 1970; Collman et al. 1976).

CO is produced in an amount of 33×10^8 metric tons per year (Conrad 1988), and its concentration

Table 1. Physiological groups of CO-utilizing bacteria.[a]

Group[b] Representative	Relation to O_2	CO as sole carbon and energy source	CO concentration tolerated %(v/v)	C assimilation
Carboxidotrophs[c]				
Pseudomonas carboxydovorans	aerobic	+	90	Ribulose bisphosphate carboxylase
Phototrophs				
Rhodocyclus gelatinosus	facultative aerobic	+	100	Ribulose bisphosphate carboxylase
Acetogens				
Clostridium thermoaceticum	anaerobic	-	30–100[d]	CODH/acetyl-CoA synthase
Methanogens				
Methanobacterium thermoautotrophicum	anaerobic	-	40	CODH/acetyl-CoA synthase
Sulfate reducers				
Desulfovibrio vulgaris	anaerobic	-	4	CODH/acetyl-CoA synthase?

[a] References are given in the text.

[b] For the recently isolated obligately CO-oxidizing *Carboxydothermus hydrogenoformans* the mechanisms of CO oxidation and utilization are not known so far (see text).

[c] Strains, capable of anaerobic denitrifying growth without the ability of profitable CO-oxidation under these conditions were described (Frunzke & Meyer 1990).

[d] Growth in the presence of 100% CO in the culture headspace was observed only after long-term adaptation to increasing amounts of CO (Kerby & Zeikus 1983).

in atmospheric air is 0.06–0.15 ppm (Seiler 1974, 1978; Heidt et al. 1980; Robinson et al. 1984).

II. Microorganisms capable of CO utilization

CO supports growth of eubacteria and archaebacteria belonging to different physiological groups. The aerobic carboxidotrophic bacteria are characterized by a respiratory chemolithoautotrophic utilization of CO as a sole source of carbon and energy. Acetogens, methanogens, sulfate-reducers, some phototrophs, and the recently isolated *Carboxydothermus hydrogenoformans* are able to grow anaerobically with CO as carbon and energy source. However, among the anaerobes, only *C. hydrogenoformans* and the phototrophs are able to grow with 100% (v/v) CO in the gas phase as sole carbon and energy source (Table 1). The physiology and genetics of the metabolic pathways involved in the utilization of CO by these different groups are summarized in the following sections and compared to each other. Only the most important references dealing with utilization of and growth on CO will be cited. For more literature concerning other aspects in the respective metabolic groups the reader is referred to the recent reviews cited below.

Studies with pure cultures also indicate that fungi (Inman & Ingersoll 1971) and algae (Chappelle 1962) can utilize CO. Since the pathways and enzymes involved in CO utilization by these organisms are not further characterized, they are not considered in this review. Also not considered is the non-utilitarian CO oxidation, which takes place in methane- and ammonia-oxidizing bacteria. The properties of the CO-oxidizing activity in these two metabolic groups were recently summarized and compared to each other (Bédard & Knowles 1989). Aerobic methanotrophic bacteria cooxidize CO as a result of the broad substrate specificity of methane monoxygenase without taking any advantage of this oxidation (Ferenci et al. 1975; Colby et al. 1979; Higgins et al. 1980; Anthony 1982). A similar situation is observed in ammonia-oxidizing bacteria, where ammonia monoxygenase fortuitously converts CO to CO_2 (Jones & Morita 1983; Jones et al. 1984).

A. Aerobic carboxidotrophic bacteria

Isolation

Aerobic bacteria isolated from garden soil, which might utilize carbon monoxide (CO) as growth substrate were first reported by Beijerinck & van Delden (1903). Since then several scientists have isolated bacteria capable of growing in the presence of air enriched with CO. The reports of Beijerinck & van Delden (1914) and Lantzsch (1922) deserve to be mentioned in this context. In the former report, two strains, *Actinobacillus oligocarbophilus* and *Actinobacillus paulotrophus*, were described which comprise a new genus *Actinobacillus*. These strains grew as white hydrophobic pellicles on the surface of culture media. In the latter report, another bacterium, *Actinomyces oligocarbophilus*, isolated from a suspension of quartz sand was described. The growth of *A. oligocarbophilus* was similar to the two strains mentioned above. However, in these as well as in several other early reports, stringent evidence for CO oxidation was not provided. In 1953, Kistner succeeded in the isolation of a motile bacterium from sewage sludge. His strain, *Hydrogenomonas carboxydovorans*, was a true carboxidotrophic bacterium capable of oxidizing CO and H_2. Fifteen years later the cultures of *H. carboxydovorans* deposited by Kistner could not be revitalized (Davis et al. 1969, 1970; Meyer & Schlegel 1978). New strains of carboxidotrophic bacteria became available mainly through the work of the research groups of Zavarzin, Hegeman, Kim, Colby and Meyer. They have been isolated from a variety of locations and samples (e.g. water, soils, sewage, compost, charcoal pile). Descriptions of individual strains and lists comparing properties of carboxidotrophic bacteria have been published earlier (Meyer & Schlegel 1983; Meyer 1989; Meyer et al. 1990). Recently, from the covering soil of a burning charcoal pile, we isolated an aerobic, thermophilic, obligately chemolithoautotrophic bacterium which was identified as *Streptomyces thermoautotrophicus* UBT1 (Gadkari et al. 1990).

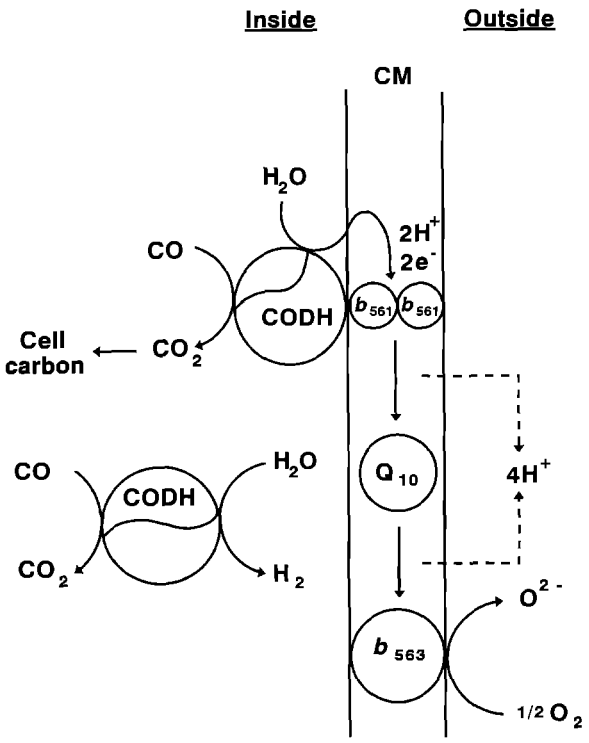

Fig. 1. Schematic model for the metabolism of CO by *Pseudomonas carboxydovorans* (data from Meyer 1989 and Meyer et al. 1990, 1992). Two molecules of CODH are shown: a membrane-associated one generating energy and a cytoplasmic one evolving hydrogen. For further explanations refer to sections II.A and III.A. Abbreviations used: CM, cytoplasmic membrane; Q_{10}, ubiquinone$_{10}$; b_{561}, b_{563}, cytochromes b_{561}, b_{563}.

S. thermoautotrophicus utilizes dinitrogen aerobically as sole nitrogen source under chemolithoautotrophic growth conditions with CO as a sole energy and carbon source or with H_2 plus CO_2 as the energy and carbon source, respectively (Gadkari et al. 1992). Obviously carboxidotrophs are cosmopolitan, and they are present in widely different areas of the world.

Physiology

Carboxidotrophic bacteria are characterized by the aerobic, respiratory, chemolithoautotrophic utilization of CO as a sole source of carbon and energy (Meyer & Schlegel 1983). The interest in the physiology and the biochemistry of CO metabolism by carboxidotrophic bacteria is reflected by the numerous reviews on this topic (Zavarzin & Nozhevnikova 1977; Colby et al. 1979; Kim & Hegeman 1983; Meyer & Schlegel 1983; Park & Hegeman 1984; Meyer & Rohde 1984; Bell et al. 1985; Meyer 1985; Meyer 1986; Meyer et al. 1986; Williams & Colby 1986; Meyer 1989; Meyer et al. 1990; Meyer et al. 1992).

Aerobic growth in the presence of up to 90% (v/v) CO in the gas phase is achieved by a CO-insensitive branch of the respiratory chain channelling electrons to a CO-insensitive terminal oxidase with high affinity for O_2 (Cypionka & Meyer 1983; Cypionka et al. 1985). Carboxidotrophic bacteria, i.e. bacteria capable of aerobic and chemolithoautotrophic growth on CO, are not closely related among themselves, but this metabolic type is established in species of different taxonomic position (Meyer & Schlegel 1983; Meyer et al. 1986). With the exception of *Pseudomonas thermocarboxydovorans* (Lyons et al. 1984), *Bacillus schlegelii* (Krüger & Meyer 1984), and *Streptomyces thermoautotrophicus* (Gadkari et al. 1990), carboxidotrophs are mesophilic organisms. In contrast to nitrifying, hydrogen-, iron-, or sulfur-oxidizing bacteria, carboxidotrophic organisms are able to generate their own CO_2 needed for assimilation. Most carboxidotrophic strains known so far feed on H_2 plus CO_2 as well. With the exception of the obligately lithoautotrophic *Streptomyces thermoautotrophicus* (Gadkari et al. 1990), carboxidotrophic bacteria also grow organoheterotrophically with a wide range of organic substrates (Meyer & Schlegel 1983).

The utilization of carbon monoxide as sole carbon and energy source by *Pseudomonas carboxydovorans* (Meyer & Schlegel 1978), the best studied carboxidotroph, is described below and depicted schematically in Fig. 1. CO is oxidized by a membrane-associated CO dehydrogenase (CODH) to CO_2 (see section III.A). Protons and electrons generated by CO oxidation are transferred to a CO-insensitive respiratory chain (Cypionka & Meyer 1983; Meyer 1989). Cytochrome b_{561} was isolated in a 2:1 complex with membrane-bound CODH and identified as the physiological electron acceptor of CODH in *P. carboxydovorans* (Jacobitz & Meyer 1989; Meyer et al. 1990).

A novel CO-insensitive, membrane-bound, low

potential *b*-type cytochrome (cytochrome b_{563}, $E^{\circ\prime} = -105\,mV$) was detected in this bacterium and identified as the terminal cytochrome oxidase with a K_m value for O_2 lower than $0.5\,\mu M$ (Cypionka et al. 1985). In the course of CO oxidation, 4 protons/oxygen atom are translocated across the cytoplasmic membrane and the resulting proton motive force can be used for the generation of ATP (Cypionka et al. 1984). CODH is not able to reduce pyridine nucleotides and therefore a pmf-driven reversed electron flow is required for the generation of reducing equivalents (Jacobitz & Meyer 1986; Meyer et al. 1986). For a more detailed review on the electron transport system and energy conservation in carboxidotrophic bacteria, see Meyer et al. (1986) and Meyer (1989). Part of the CO_2 formed upon CO oxidation is assimilated *via* ribulose bisphosphate carboxylase and the reductive pentose phosphate cycle (Meyer & Schlegel 1978; Futo & Meyer 1986).

The stoichiometry of aerobic carboxidotrophic growth was determined to be:

$$2.19\ CO + O_2 \rightarrow 1.83\ CO_2 + 0.36\ \text{cell C}$$

This means that about 16% of the CO_2 generated in order to gain energy is assimilated under optimal conditions. Under unfavorable conditions, this value can drop to only 2% (Meyer & Schlegel 1983; Meyer 1989). Doubling times for growth with CO were 3–4 h with strains of *Bacillus schlegelii* (Krüger & Meyer 1984) and 18–20 h with *P. carboxydovorans* (Meyer & Schlegel 1978).

Formerly, the metabolism of carboxidotrophic bacteria was considered to be strictly aerobic. Calculations of the free energy changes for the bacterial utilization of CO or H_2 with oxidized nitrogen compounds serving as electron acceptors did not exclude the possibility of nitrate reduction or denitrification by carboxidotrophic bacteria (Meyer & Rohde 1984). A strain of *Rhizobium japonicum* was reported to grow on CO with nitrate as the terminal electron acceptor (Gunatilaka et al. 1983). On the other hand, it was known that CO inhibits the reduction of N_2O in intact cells of *Alcaligenes sp.* (Matsubara & Mori 1968) and in extracts of *Paracoccus denitrificans* (Kristjansson & Hollocher 1980).

A study on nitrate-respiring and denitrifying capabilities in our laboratory revealed that *Pseudomonas carboxydoflava, Pseudomonas carboxydohydrogena, Pseudomonas compransoris,* and *Pseudomonas gazotropha* can utilize nitrate, nitrite, nitric oxide and nitrous oxide in the absence of oxygen as terminal electron acceptors for respiration (Frunzke & Meyer 1990). Denitrification occurs only with organic C-substrates such as pyruvate, lactate, or nutrient broth. Under these conditions CO cannot be used as a source of electrons or carbon. Its presence inhibits denitrifying growth by about 50% (Frunzke & Meyer 1990).

Genetics

The ability to use CO as a sole source of carbon and energy requires the presence of several genes encoding the special enzymes or regulatory elements involved in the process. Structural genes encoding key enzymes in the metabolism of CO are *coxL, M,* and *S* (CODH), *cfxL* and *S* (large and small subunit of ribulose bisphosphate carboxylase), *cfxP* (phosphoribulokinase), *pac* (primary electron acceptor for CODH), and *tox* (CO-insensitive terminal oxidase). Not essential for carboxidotrophic growth, but important to those strains capable of hydrogen-dependent autotrophic growth, are genes encoding an uptake hydrogenase (*hox*). In plasmid-harbouring strains the genes mentioned are either encoded on the plasmid(s) or the chromosome exclusively, or they are duplicated and present on both types of genetic elements.

Many carboxidotrophic bacteria were found to harbour 1,2 or 3 plasmids (Gerstenberg et al. 1982; Kraut & Meyer 1988). These plasmids ranged in size from 45 to 558 kb. Small plasmids of approximately 3.5 kb were detected in *Azotobacter* sp. and *Azomonas* sp. (Park & Hegeman 1984; Kwon & Kim 1985).

The best studied carboxidotroph, *Pseudomonas carboxydovorans*, harbours the 128 kb plasmid pHCG3 (Gerstenberg et al. 1982; Kraut & Meyer 1988). A set of mutants with defects in autotrophic growth was isolated and characterized (Meyer 1985; Kraut & Meyer 1988). A mutant cured of

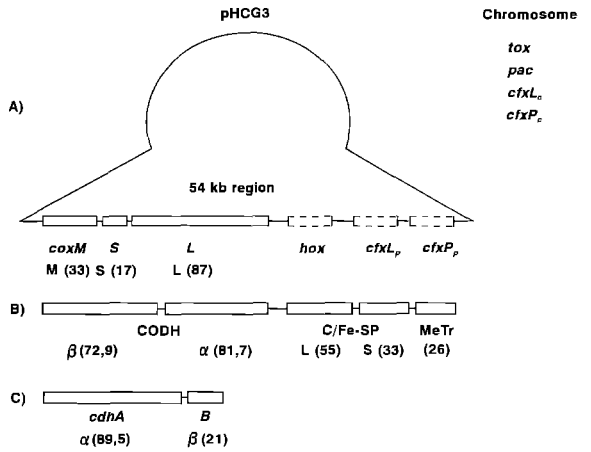

Fig. 2. Organization of genes encoding CODH and related enzymes. (A) Localization and organization of genes encoding CODH and other enzymes essential for chemolithoautotrophic metabolism in *Pseudomonas carboxydovorans*. The relative distance and organization of hydrogenase- (*hox*), ribulose bisphosphate carboxylase- ($cfxL_p$), and phosphoribulokinase- ($cfxP_p$) encoding genes with respect to each other and to the CODH encoding genes (*cox*) is presently not known. (B) Organization of the genes encoding CODH, the corrinoid/Fe-S protein (C/Fe-SP) subunits, and methyltransferase (MeTr) in *Clostridium thermoaceticum*. (C) Organization of the genes encoding CODH subunits in *Methanothrix soehngenii*.

The numbers in brackets give the molecular weight of the proteins in kDa; further explanations in the text.

pHCG3 (OM5–12) as well as mutants with deletions in pHCG3 were isolated. Mutant OM5–24 had suffered a deletion of 15 kb in pHCG3 (Δ^1pHCG3) and lost the ability of hydrogen-dependent autotrophic growth, whereas OM5–29 had suffered a 54 kb deletion in pHCG3 (Δ^2pHCG3), and as a result, had lost both the capacity for hydrogen- dependent and CO-dependent growth (Kraut & Meyer 1988).

Employing oligonucleotide probes derived from the N-terminal amino acid sequences of the purified CODH subunits, the *cox* structural genes could be identified on large plasmids in plasmid-harbouring strains. In such strains, the *cox* genes are located exclusively on the plasmid; no homologous genes could be detected on the chromosome. The *cox* structural genes were shown to be localized on pHCG3 in *P. carboxydovorans* and to be absent from Δ^2pHCG3 in mutant OM5–29 (Kraut et al. 1989; Hugendieck & Meyer 1992). Recently, the *cox* structural genes of *P. carboxydovorans* were cloned on a 4.5 kb restriction fragment of plasmid pHCG3. Expression of the fragment-encoded polypeptides in *Escherichia coli* revealed proteins corresponding in size to CoxS and CoxL, which also cross-reacted with antibodies raised against the respective enzyme subunits. CoxM was not expressed in *E. coli* but DNA-sequencing of a region upstream of *coxS* revealed the 5′ end of the *coxM* gene. The 5′ coding regions of *coxS* and *coxL* were also localized by DNA sequencing. This revealed a gene cluster with the order *coxMSL* (Fig. 2, Kraut et al. 1992).

The structural genes encoding CODH of *Pseudomonas thermocarboxydovorans* were cloned and expressed on a 4 kb fragment of chromosomal DNA. Although the three subunits were expressed, no active CODH was detectable in *E. coli*. As discussed by the authors, this could be due to the lack of accessory genes required for the formation of an active enzyme (Black et al. 1990). The *cox* structural genes from *P. thermocarboxydovorans* are probably also arranged in the order *coxMSL* (O'Reilly et al. 1992).

Molybdenum in CODH is bound to a novel organic component of the cofactor, molybdopterin cytosine dinucleotide (MCD, see section III.A). The biosynthesis of molybdenum cofactors is currently under investigation and was extensively reviewed by Hinton & Dean (1990). In *E. coli*, 3 classes of chlorate-resistant mutants (*chlA, chlB*, and *chlE*) are specifically defective in the biosynthesis of molybdopterin cofactors. Therefore, screenings for mutants resistant to chlorate or incapable of CO-dependent growth would also yield mutants defective in Mo-cofactor biosynthesis. In the course of such screenings, one out of 61 mutants from *P. carboxydovorans* was found to show the respective phenotype (Meyer 1985). The further characterization of this mutant (OM5–41) with respect to its plasmid-content, expression of the *cox* genes, and physiology is the subject of current research.

Employing heterologous gene probes, *cfxL* and *cfxP* genes were detected on the chromosome as well as on plasmids in plasmid-harbouring strains (Hugendieck & Meyer 1991). This suggested duplicated genes. In the mutant OM5–29 the pHCG3-

encoded $cfxL_p$ and $cfxP_p$ genes were deleted, thus indicating their location within the 54 kb region (Fig. 2).

The pHCG3-cured mutant OM5–12 and the deletion mutants OM5–24 and OM5–29 have lost the ability to grow autotrophically on H_2. The absence of hydrogenase activity in OM5–24 and OM5–29 shows that either the hydrogenase structural genes or genes necessary for hydrogenase formation reside within a region of pHCG3 deleted from both Δ^1pHCG3 and Δ^2pHCG3 (Fig. 2, Kraut & Meyer 1988).

Recently, we purified the hydrogenase of *P. carboxydovorans* and determined the N-terminal amino acid sequence of the two subunits (Santiago & Meyer 1992). Oligonucleotide probes derived from these sequences will allow the localization of the *hox* structural genes in this organism.

Cytochrome b_{561} was shown to serve as the electron acceptor for CODH in *P. carboxydovorans* (Jacobitz & Meyer 1989; Meyer et al. 1990). The cytochrome is a monomer of M_r = 55 000, and hence a structural gene *(pac)* of at least 1500 bp can be expected. During growth on CO with O_2 as terminal electron acceptor, *P. carboxydovorans* uses cytochrome b_{563} as an alternative CO-insensitive terminal oxidase. Since cytochrome b_{563} is an essential component in carboxidotrophic metabolism, its biochemical characterization and the identification of its structural genes (*tox*) have been envisaged. Results obtained with the pHCG3-cured mutant OM5–12 indicate that the *pac* and the *tox* genes are both located on the chromosome (Fig. 2).

Since carboxidotrophic bacteria belong to different taxonomic groups while the CODH enzymes are conserved among these organisms, it was considered that the *cox* genes might have been distributed by horizontal gene transfer (Kraut et al. 1989). Hydrogenase, phosphoribulokinase, and ribulose bisphosphate carboxylase also were shown to be plasmid-encoded in plasmid-harbouring carboxidotrophic bacteria. It thus is tempting to assume the involvement of an 'autotrophy' plasmid in the distribution of these genes. This view is supported by the finding that all the traits mentioned above are localized within a 54 kb region of pHCG3

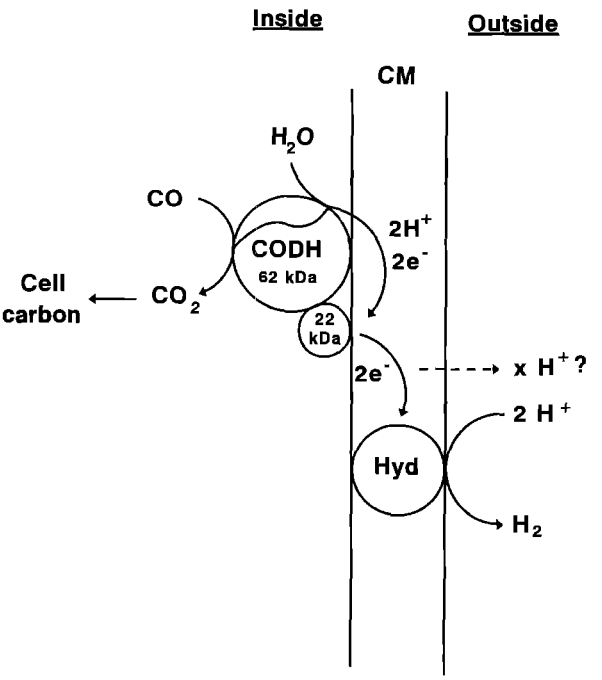

Fig. 3. Schematic representation of CO utilization by phototrophic bacteria. The model shows the present understanding of carbon utilization and energy generation by *Rhodospirillum rubrum* (Ensign & Ludden 1991). For further explanations refer to sections II.B and III.B. Abbreviations: Hyd, CO-insensitive hydrogenase.

(Kraut et al. 1989; Meyer et al. 1990; Hugendieck & Meyer 1991, 1992). To date, no other traits encoded by these plasmids are known.

B. Phototrophic bacteria

When phototrophic bacteria had been shown to tolerate CO (Hirsch 1968), it was demonstrated that *Rhodocyclus (Rhodopseudomonas) gelatinosus* (Uffen 1976; Dashekvicz & Uffen 1979) and *Rhodospirillum rubrum* (Uffen 1981) are able to grow anaerobically in the dark with CO as the sole source of carbon and energy. *R. gelatinosus* grows in the presence of 100% (v/v) CO in the gas phase and reaches generation times of 6.7 h (Uffen 1976). The oxidation of CO yields CO_2 and H_2 according to the following equation:

$$CO + H_2O \rightarrow CO_2 + H_2$$

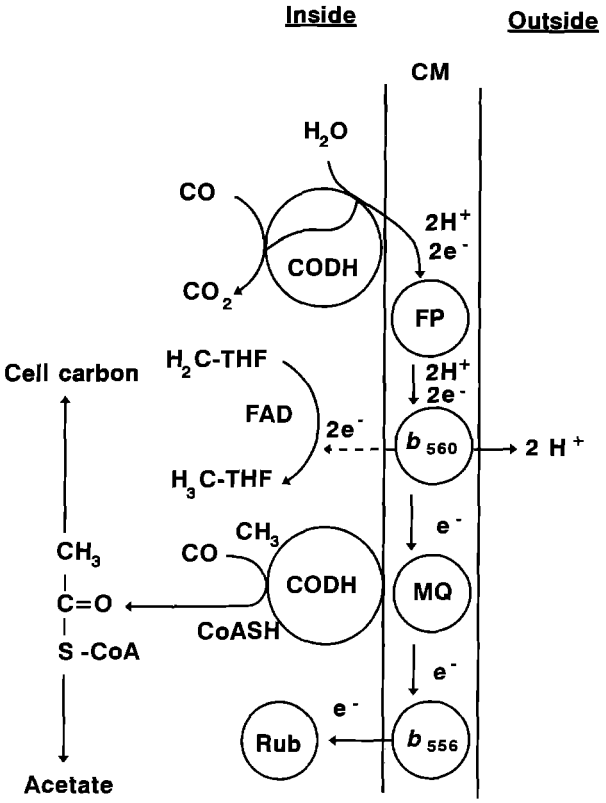

Fig. 4. Scheme of CO utilization by anaerobic acetogenic bacteria (according to Hugenholtz & Ljungdahl 1989, modified). The model represents the present understanding of CO metabolism in *Clostridium thermoautotrophicum*. Two molecules of CODH/acetyl-CoA synthase are shown: one oxidizing CO and generating energy, the other one fixing CO into acetyl-CoA. For further explanations refer to sections II.D and III.C. Abbreviations: CODH, CODH/acetyl-CoA synthase; FP, flavoprotein; b_{560}, b_{556}, cytochromes b_{560}, b_{556}; MQ, menaquinone; Rub, rubredoxin; H_2C-, H_3C-THF, methylene-, methyl-tetrahydrofolate.

Part of the CO_2 generated by this reaction is fixed by the enzymes of the Calvin cycle (Dashekvicz & Uffen 1979). The CO-oxidizing system is induced by the presence of CO and is membrane-associated (Uffen 1983, Wakim & Uffen 1983, Bonam & Ludden 1987, Ensign & Ludden 1991). The CODH catalyzing this reaction in *R. rubrum* was purified and characterized (Bonam & Ludden 1987; Ensign & Ludden 1991), and its properties are discussed in section III.B.

Electrons and protons derived from CO oxidation are transferred to a CO-inducible and CO-insensitive hydrogenase (Bonam et al. 1989) and this transfer is probably coupled to the generation of a proton motive force leading to the formation of ATP (Fig. 3, Ensign & Ludden 1991).

C. Thermocarboxydovorans hydrogenoformans

The recently isolated *Thermocarboxydovorans hydrogenoformans* (Svetlichny et al. 1991) is a thermophilic, Gram-positive, anaerobic eubacterium and was characterized as obligately carboxidotrophic. It grows in the presence of 100% (v/v) CO in the gas phase, producing equimolar amounts of H_2 and CO_2; methane or acetate are not formed. In this respect, it compares to the phototrophic bacteria. The bacterium is neither capable of H_2-dependent autotrophic growth nor of heterotrophic growth with one of the organic substrates tested. The doubling time under optimal growth conditions was 2 h and the CO oxidation rate 7.4 μmol CO · ml culture^{-1} · h^{-1}. Up to now, there is no information available about the enzyme system oxidizing CO, the electron acceptor for this reaction or the route of CO_2 fixation in this organism.

D. Acetogenic bacteria

Acetogenic bacteria are obligately anaerobic eubacteria forming acetate from CO_2 or organic substrates (for recent reviews see Fuchs 1986; Diekert 1990; Wood 1991; Wood & Ljungdahl 1991). *Clostridium thermoaceticum* and *Clostridium formicoaceticum* have been shown to contain CO-oxidizing activity leading to the formation of acetate (Diekert & Thauer 1978). The enzyme catalyzing acetogenesis from CO in *C. thermoaceticum* was purified and characterized (Drake et al. 1980). CO is fixed via CODH/acetylCoA synthase (see section III. C). The enzyme in acetogens normally reduces CO_2 to CO which in turn is fixed as a carbonyl group to the protein. The enzyme then forms acetyl-CoA from an enzyme-bound methyl group, the enzyme-bound carbonyl, and a bound CoA group. When present in the medium, CO is bound directly to CODH/acetyl-CoA synthase and either oxidized

to CO_2 or directly incorporated into acetyl-CoA (Fig. 4, Wood 1991; Wood & Ljungdahl 1991). The primary electron acceptor used by CODH in CO oxidation was assumed to be rubredoxin, because it was by far the best naturally occurring electron acceptor for the enzyme (Ragsdale et al. 1983). The oxidation of CO is coupled to the translocation of protons across the cell membrane, and the proton gradient can be used for the uptake of amino acids (Diekert et al. 1986; Hugenholtz & Ljungdahl 1989) and probably for the generation of ATP (Diekert et al. 1986; Hugenholtz et al. 1987; Hugenholtz & Ljungdahl 1989). A model of the electron carriers involved in energy conservation was proposed (Hugenholtz & Ljungdahl 1989; Wood & Ljungdahl 1991). In this scheme, a yet unidentified flavoprotein serves as the primary electron acceptor and electrons are then channelled *via* cytochromes b_{560} and b_{556} to reduce methylene-tetrahydrofolate and rubredoxin (Fig. 4).

The utilization of CO by acetogens therefore yields energy, cell material, acetate, and CO_2. *C. thermoaceticum* was shown to grow with CO as electron donor (Kerby & Zeikus 1983) and it has been demonstrated that CO oxidation was accompanied by evolution of H_2 (Martin et al. 1983). *C. thermoaceticum* could grow chemolithotrophically in a defined medium with 30% (v/v) CO in the culture headspace as carbon and energy source (Daniel et al. 1990). The stoichiometry of CO utilization was:

$$6.8\ CO \rightarrow CH_3COOH + 3.5\ CO_2 + 0.4\ \text{cell C} + 0.9\ \text{unrecovered C}$$

Hence, the utilization of CO yielded only about 51% CO_2, the rest was fixed into cell material (6%) and acetate (29%).

Acetogenium kivui was not able to grow with CO as electron donor (Daniel et al. 1990), but CO was incorporated into cell material and acetate during H_2-dependent growth (Yang & Drake 1990). This indicates that the electrons derived from the oxidation of CO could not be coupled to energy conservation in this organism.

Among the acetogenic bacteria, *Peptostreptococcus productus* (Lorowitz & Bryant 1984; Geerligs et al. 1987; Ma et al. 1987; Ma et al. 1991) is the species tolerating the highest CO concentrations. Strains of this species were described to grow in the presence of 90% (v/v) (Lorowitz & Bryant 1984) and 50–70% (v/v) CO (Geerligs et al. 1987) in the gas phase, respectively. Furthermore, with reported doubling times of 1.5 and 3 h (Lorowitz & Bryant 1984; Geerligs et al. 1987), it is the acetogen showing the fastest growth on CO.

Genetics

The genes encoding the key enzymes in acetyl-CoA synthesis in *Clostridium thermoaceticum* were cloned on a 10 kb genomic fragment (Roberts et al. 1989). The genes coding for the single subunit of methlytransferase, the two subunits of CODH/acetyl-CoA synthase and the two subunits of the corrinoid/Fe-S protein were found to be clustered within this fragment (Fig. 2). Heterologous expression in *E. coli* yielded an active enzyme only in the case of methyltransferase. Both CODH/acetyl-CoA synthase and the corrinoid/Fe-S protein subunits were also formed, but did not correctly assemble and hence no active enzymes were found (Roberts et al. 1989).

The primary structure of the two CODH/acetyl-CoA synthase subunits (α = 81,7 kDa, β = 72,9 kDa) was deduced from the respective gene sequences (Morton et al. 1991) and is discussed in section III.C.

E. Methanogenic bacteria

Methanogenic bacteria are obligately anaerobic archaebacteria forming methane from CO_2, other C_1-compounds, or acetate (for recent reviews see Thauer et al. 1989; Bhatnagar et al. 1991). Growth of a pure culture of *Methanobacterium thermoautotrophicum* with CO was first demonstrated by Daniels et al. (1977). CO is used by this bacterium as an electron donor reducing F_{420}. This electron acceptor is also used during growth with H_2 as an electron donor, but at 100-fold higher rates. Hence, the growth rate with CO as electron donor was maximal 1% of that observed with H_2. Growth was best in the presence of 30% (v/v) CO in the gas

phase and ceased when the CO concentration reached 60% (v/v). H_2-dependent growth and methanogenesis were also affected by the presence of CO and decreased to 10% when 40% (v/v) CO were present (Daniels et al. 1977).

CO_2, generated by the oxidation of CO, then is used as the electron acceptor leading to the formation of methane and the generation of energy for growth according to the following equation (Daniels et al. 1977):

$$4\ CO + 2\ H_2O \rightarrow 3\ CO_2 + CH_4$$

The enzyme catalyzing CO oxidation in methanogens is also a CODH/acetyl-CoA synthase (see section III.C) and serves the same metabolic function as in acetogens: the synthesis of acetyl-CoA from two C_1-precursors, one of which is an enzyme-bound carbonyl. This carbonyl group is derived from CO, which normally is generated by the enzyme through CO_2 reduction.

The presence of 5% (v/v) CO in the gas phase during growth of *Methanobacterium thermoautotrophicum* with H_2 plus CO_2 led to the incorporation of 15% of the CO-carbon into the C_2 of alanine, which originates from the carboxyl group of acetate (Stupperich et al. 1983).

In acetoclastic methanogenic bacteria the reverse reaction takes place: CODH/acetyl-CoA synthase catalyzes the cleavage of acetyl-CoA leading to a disproportionation to CH_4 and CO_2 (recently reviewed by Thauer et al. 1989). The energy derived from CO oxidation to CO_2 and H_2 was shown to be coupled to the production of ATP in the acetoclastic *Methanosarcina barkeri* (Bott et al. 1986) and ferredoxin was demonstrated to mediate the electron flow from CODH to a membrane-bound hydrogenase in the acetoclastic *Methanosarcina thermophila* (Terlesky & Ferry 1988). Whether CO-dependent H_2 production in these organisms plays a role in the reduction of methyl-coenzyme M or whether it is the result of reducing-equivalent regeneration still needs further elucidation as well as the characterization of the electron carriers involved in this process.

In summary, the utilization of CO also yields energy, CO_2, and cell material in methanogens, but in contrast to acetogens the main product of CO_2 reduction is CH_4 instead of acetate. A difference is also the higher sensitivity towards CO which was assigned to inhibitory effects on hydrogenase (Bott et al. 1986) and obviously CO is not a good electron donor for methanogens.

Genetics

The genes encoding the two subunits of CODH/acetyl-CoA synthase from the acetoclastic *Methanothrix soehngenii* were cloned on a 4.8 kb *Hin*dIII fragment and sequenced (Eggen et al. 1991). The *cdhA* gene for the larger subunit (89.5 kDa) precedes the *cdhB* gene encoding the smaller subunit (21 kDa) (Fig. 2). The primary structure of the deduced proteins is discussed in section III.C.

F. Sulfate-reducing bacteria

Sulfate reducers are obligately anaerobic bacteria using sulfate as terminal electron acceptor and capable of growing autotrophically (for review see Fauque et al. 1991). The oxidation of CO by cell extracts of sulfate-reducing bacteria was demonstrated by Yagi and coworkers (Yagi 1958, 1959; Yagi & Tamiya 1962). The CODH enzyme catalyzing the reaction in *Desulfovibrio desulfuricans* (section III.C) was partially purified and characterized by Meyer & Fiebig (1985). Schauder et al. (1986) proposed a pathway for acetate degradation in sulfate-reducers involving a CODH/acetyl-CoA synthase, which could be demonstrated in *Desulfobacterium autotrophicum* (Schauder et al. 1989). Growth of *Desulfovibrio barsii* on formate and CO_2 in the presence of 1.5% (v/v) CO resulted in the incorporation of about 15% of the CO-carbon into the amino acids alanine, aspartate, and glutamate (Jansen et al. 1984).

Desulfovibrio vulgaris strain Madison was shown to utilize CO as electron donor when present at a concentration of up to 4% (v/v) in the gas phase. CO consumption was accompanied by the production of H_2 which in turn was consumed. Concentrations of 4.5% (v/v) CO and higher were inhibitory to growth probably due to inhibition of hydrogenase or electron carriers involved in the reduction

of sulfate (Lupton et al. 1984). *Desulfotomaculum* species were reported to grow slowly with CO as the only energy source at concentrations of 5–20% (v/v) in the culture headspace. CO concentrations higher than 20% (v/v) inhibited growth (Klemps et al. 1985). The pathway of conservation of CO-derived energy in sulfate reducers still needs to be elucidated.

III. The enzymes: CO dehydrogenases (CODH)

CODH enzymes share the ability to oxidize CO to CO_2, but comparing their biochemical properties, it seems that in nature at least three different systems have evolved for the utilization of CO: i) the molybdenum-containing hydroxylase of aerobic carboxidotrophs, ii) the nickel-containing enzyme of phototrophic bacteria, and iii) the nickel-containing CODH/acetyl-CoA synthase of acetogenic, methanogenic, and sulfate-reducing anaerobes. Common to all these electron-transferring enzymes is the presence of Fe-S centers.

Whereas all enzymes are quite well characterized on the protein level, only those of two carboxidotrophic, of an acetogenic, and of a methanogenic bacterium are now amenable to molecular analysis, since their structural genes have been cloned. Some biochemical data of well-studied enzymes in the respective groups are compared in Table 2.

A. Molybdenum-containing CODH

The oxidation of CO to CO_2 in carboxidotrophic bacteria is catalyzed by CODH at the expense of water:

$$CO + H_2O + A(\text{oxidized}) \rightarrow CO_2 + AH_2(\text{reduced})$$

CODH therefore is a hydroxylase and termed CO:acceptor oxidoreductase (Meyer & Schlegel 1983; Kim & Hegeman 1983). It can utilize artificial electron acceptors (A) with $E^{\circ\prime}$ ranging from -34 mV to 217 mV and the membrane-bound cytochrome b_{561} is assumed to be the physiological electron acceptor (Meyer et al. 1990). CODH is a molybdoiron-sulfur flavoprotein and molybdenum in CODH is present in the molybdenum cofactor (Krüger & Meyer 1986, 1987) containing molybdopterin cytosine dinucleotide (MCD) as the orga-

Table 2. Properties of some well characterized CO dehydrogenases.

Organism	Subunits	M_r (kDa)	Active enzyme				
			Subunit structure	M_r (kDa)	Metal atoms	Cofactors	K_m value (μM CO)
Pseudomonas[a]	L	86	$L_2M_2S_2$ or	300	2 Mo	2 Mo-MCD	53
carboxydovorans	M	34	$(LMS)_2$		8 Fe	2 FAD	
	S	17				2 (2Fe-2S)I	
						2 (2Fe-2S)II	
Rhodospirillum[b]	α	62	α	62	1 Ni	(4Fe-4S)?	110
rubrum					8 Fe	(2Fe-2S)?	
Clostridium[c]	α	81.7	$\alpha_3\beta_3$ or	440	6 Ni	6 (4Fe-4S)	3,000
thermoaceticum	β	72.9	$(\alpha\beta)_3$		3–9 Zn	3 (2Fe-2S)	
					33 Fe	3 (Fe-4S)	
Methanothrix[d]	α	88.1	$\alpha_2\beta_2$ or	190	2 Ni	2 (4Fe-4S)	700
soehngenii	β	21	$(\alpha\beta)_2$		18 Fe	2 (6Fe-6S)	

Data are taken from:

[a] Meyer 1982; Bray et al. 1983; Meyer & Rajagopalan 1984; Meyer & Rohde 1984.

[b] Bonam et al. 1984; Bonam & Ludden 1987; Ensign et al. 1989; Ensign & Ludden 1991.

[c] Ragsdale et al. 1983; Lindahl et al. 1990; Morton et al. 1991.

[d] Jetten et al. 1989, 1991; Eggen et al. 1991.

nic component (Johnson et al. 1990). Moreover, the enzymes contain FAD and two types of 2Fe-2S centers (Table 2). CODH enzymes from carboxidotrophic bacteria are composed of three different subunits (L = 87, M = 30, S = 17 kDa approximately) and the K_m values for CO are in the range of 0.4–63 μM (Meyer 1982; Bray et al. 1983; Meyer & Rajagopalan 1984; Krüger & Meyer 1986; Meyer 1989; Fuchs et al. 1992).

Immunological localization studies revealed that during exponential growth, 87% of the enzyme is associated with the inner aspect of the cytoplasmic membrane in *Pseudomonas carboxydovorans* (Rohde et al. 1984, 1985). The membrane-associated enzyme is distributed into a tightly membrane-bound and a more easily removable fraction (Jacobitz & Meyer 1989). The tightly bound fraction can be reversibly solubilized *in vitro* using zwitterionic or non-ionic detergents. Reconstitution of depleted membranes was achieved by the action of divalent cations, e.g. Ca^{2+}, Mg^{2+}, Mn^{2+}, (Jacobitz & Meyer 1989). At the end of the exponential growth phase, CODH loses contact to the inner aspect of the cytoplasmic membrane and largely (more than 50%) becomes a cytoplasmic enzyme (Rohde et al. 1984, 1985). This loss of association with its natural electron acceptor coincides with a reduction in CO-dependent respiration rates (Rohde et al. 1985) and with hydrogen-evolving activity which could be shown to be catalyzed by purified CODH (Santiago & Meyer 1992). The reversible reaction, H_2 oxidation, is also catalyzed and the K_m value for H_2 was determined to be 5 mM (Meyer et al. 1990). A reasonable explanation for this finding is that, after having lost contact to its physiological electron acceptor, CODH delivers the electrons to protons and generates hydrogen (Fig. 1).

Recently, we reported on the isolation and characterization of CODH from the Gram-positive *Streptomyces thermoautotrophicus* (Fuchs et al. 1992). This enzyme transfers electrons to reduced viologen dyes (Gadkari et al. 1990; Fuchs et al. 1992), a property which was reported earlier only for CODH from anaerobic bacteria. CODH from *S. thermoautotrophicus* exhibits the lowest K_m value for CO (0.4 μM) reported so far (Fuchs et al. 1992). High affinity for the substrate is a characteristic of the enzymes from thermophiles, probably because of the reduced solubility of CO at higher temperatures. The enzyme from the thermophilic *Pseudomonas thermocarboxydovorans* exhibits a K_m value of 0.5 μM CO (Bell et al. 1985).

The genes *coxS*, *coxM*, and *coxL* encoding the three subunits of CODH from *P. carboxydovorans* were successfully cloned in our laboratory (Kraut et al. 1992) and sequencing is in progress. Although not yet completed, the first deduced amino acid sequences show that similarity exists to xanthine dehydrogenase, another molybdenum-containing hydroxylase (Amaya et al. 1990). Similar results are found with the sequences of Cox proteins from *P. thermocarboxydovorans* (O'Reilly et al. 1992). But at the time, no good homologies to other bacterial molybdoenzymes are found. Similarities of CODH from carboxidotrophic bacteria to xanthine dehydrogenase are evident on the basis of biochemical data as discussed by Meyer et al. (1986). Completion of the primary structures will help to understand the function of the subunits in enzyme catalysis and binding of the different cofactors.

With the limited sequence data available (N-terminal amino acid sequences and partial gene sequences) no significant homologies to either one of the two CODH/acetyl-CoA synthase sequences (Eggen et al. 1991; Morton et al. 1991) were found. This is not surprising, since i) even the two CODH/acetyl-CoA synthases, although functionally equivalent, show no extended regions of similarity (Morton et al. 1991), ii) the enzymes from aerobes and anaerobes differ completely in subunit composition, affinity, metal (molybdenum *versus* nickel) and cofactor content, iii) the only features shared by both types of enzymes is the oxidation of CO to CO_2 and the presence of Fe-S centers.

B. Nickel-containing Rhodospirillum rubrum CODH

CODH from *R. rubrum* was first purified and characterized as a monomeric enzyme with a molecular weight of 62 kDa (Bonam & Ludden 1987). This protein is capable of CO oxidation in the presence of artificial electron acceptors and contains 1 nickel

and 8 iron atoms. The K_m value for CO was determined to be 110 μM with methyl viologen as electron acceptor (Bonam et al. 1984). The iron is present either in 4Fe-4S or in 2Fe-2S centers (Bonam & Ludden 1987). Nickel has been demonstrated to be specifically required for the transfer of electrons from CO to the Fe-S center(s) in this protein (Ensign et al. 1989). In a more recent study, it was found that the enzyme consists of two subunits, when heat treatment is avoided to release it from the chromatophore membrane (Ensign & Ludden 1991). The second subunit (22 kDa) is present in equimolar amounts and contains 4 Fe, probably present in a 4Fe-4S center. This Fe-S center could only be reduced by CO in the enzyme consisting of both subunits; in the isolated 22 kDa subunit it could not be reduced by CO. The small subunit was required for electron transfer from CO to the CO-inducible hydrogenase in isolated membranes (Ensign & Ludden 1991) and the present understanding of the enzymes function is as follows: CO is bound and oxidized at the 62 kDa subunit and the electrons are transferred to the Fe-S center(s) in this subunit. They in turn reduce the Fe-S center in the 22 kDa subunit. This subunit is necessary to mediate the electron flow to the CO-inducible, membrane-bound hydrogenase, which produces hydrogen (Fig. 3). Whether further membrane-bound electron-carriers are involved in this transfer and whether a proton-motive force is generated in the course of this reaction remains to be investigated.

Using polyclonal antibodies raised against the *R. rubrum* enzyme, Bonam & Ludden (1987) demonstrated the absence of immunological cross-reaction with the enzymes from *C. thermoaceticum* and *Methanosarcina barkeri*. The enzyme from *R. rubrum* did also not cross-react with antibodies raised against *M. barkeri* CODH/acetyl-CoA synthase. Thus, CODH from phototrophs seems to be immunologically unrelated to those of anaerobic methanogenic or acetogenic bacteria.

C. Nickel-containing CODH/acetyl-CoA synthases

Based on their ability to oxidize CO to CO_2, the nickel-containing enzymes from anaerobic acetogenic, methanogenic, and sulfate-reducing bacteria were discovered and therefore called CODH (Yagi 1958; Daniels et al. 1977; Diekert & Thauer 1978). But currently these enzymes are mostly designated CODH/acetyl-CoA synthases defining their physiological function in the cell (Wood 1991). However, these enzymes are commonly assayed in the CO-oxidizing reaction using artificial electron acceptors, e.g. methyl viologen. The CO-oxidizing activity was shown to be the most stable one among the different reactions catalyzed by the enzyme from *C. thermoaceticum* (Ragsdale & Wood 1985). Generally CODH/acetyl-CoA synthase enzymes from anaerobes are very sensitive towards oxygen and only the enzymes from *Desulfovibrio vulgaris* (Meyer & Fiebig 1985), *Desulfobacterium autotrophicum* (Schauder et al. 1989), and *Methanothrix soehngenii* (Jetten et al. 1989) are reported to be stable in the presence of oxygen.

Although these enzymes serve the same physiological function in the autotrophic pathway of acetogens, methanogens, and sulfate-reducers, namely the formation of acetyl-CoA from two C_1-precursors and CoASH, they are diverse in several respects (Wood & Ljungdahl 1991). In acetoclastic methanogens and most acetoclastic sulfate-reducers the enzyme fulfils a catabolic role in cleaving acetyl-CoA to CO_2 plus CH_4 or CO_2, respectively (Thauer et al. 1989).

Obvious differences are the subunit structure and the metal content (Table 2). In acetogens, two subunits with quite similar molecular weight (α = 82, β = 73 kDa approximately) are present, while in methanogens there is a large and a small subunit (α = 90, β = 20 kDa approximately).

The primary structure of two different CODH/acetyl-CoA synthase enzymes has been deduced from the respective gene sequences (Eggen et al. 1991; Morton et al. 1991). The proteins from the acetoclastic methanogen *M. soehngenii* and the acetogen *C. thermoaceticum* are quite different. As discussed by Morton et al. (1991) there are two small regions of homology between the β subunit of *C. thermoaceticum* and the α subunit of *M. soehngenii*. An amino acid sequence containing a tryp-

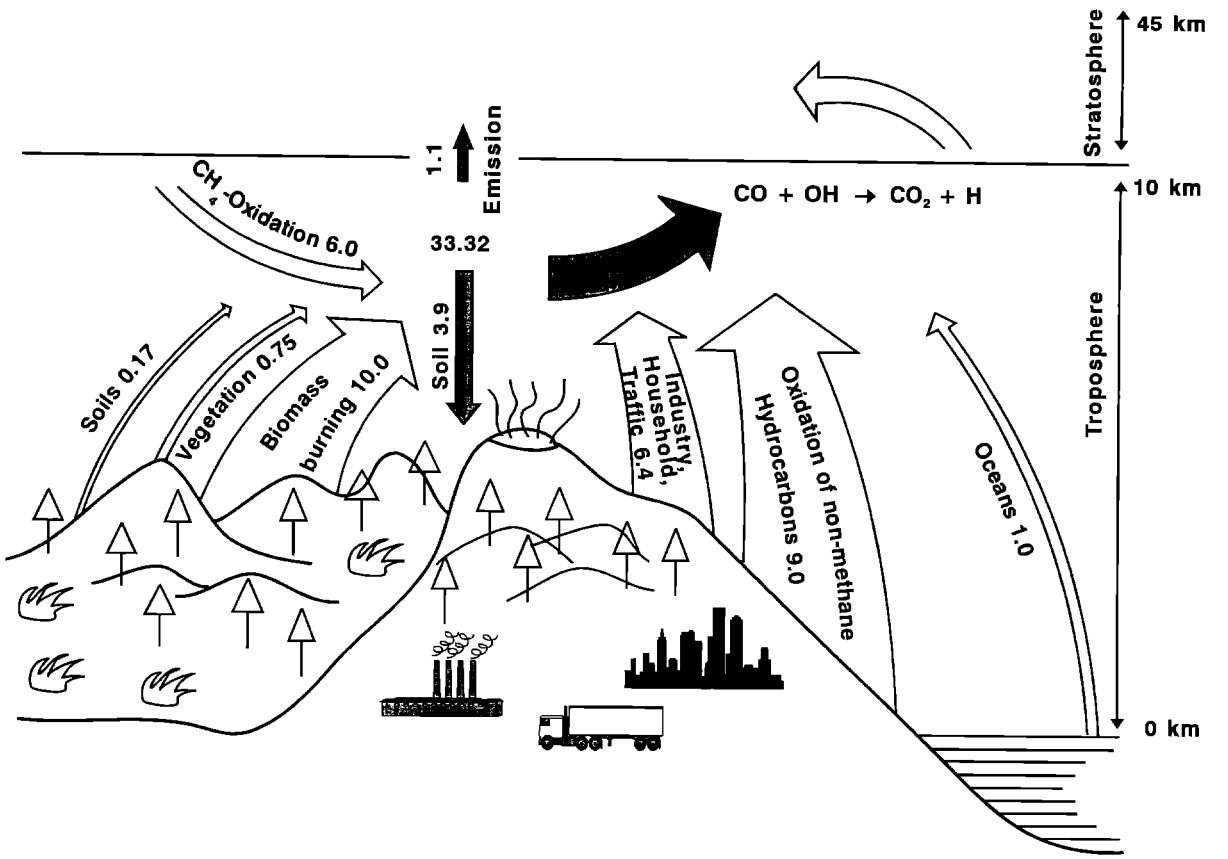

Fig. 5. Scheme of CO sources and sinks. The different sources contributing to the total amount of 33.32 · 10^8 metric tons CO per year as well as CO sinks (shaded arrows) are shown. Data are taken from Conrad (1988) and the numbers give the amount of CO in 10^8 metric tons.

tophan within the α subunit of *C. thermoaceticum* was proposed to participate in CoASH binding. In the sequence of the α subunit from *M. soehngenii* a region with homology to acyl-CoA oxidases and a cysteine-rich region with homology to ferredoxins was detected. Two cysteine-rich regions in each of the subunits of the *C. thermoaceticum* enzyme were proposed to be involved in the formation of Fe-S centers.

EPR and EXAFS studies with the *C. thermoaceticum* enzyme revealed the presence of at least two 4Fe-4S centers per αβ dimer, one of which forms a Ni-Fe-C complex when CO is bound (Bastian et al. 1988; Lindahl et al. 1990). EPR data on the enzyme from *M. soehngenii*, which is unusual in its insensitivity towards oxygen and cyanide, were interpreted to derive from one to two ferredoxin-like 4Fe-4S clusters and a 6Fe-6S cluster/αβ dimer (Jetten et al. 1991). Whereas CODH/acetyl-CoA synthases from acetogens exchange CO with the carbonyl group of acetyl-CoA (Wood & Ljungdahl 1991), the enzyme from *M. soehngenii* is the only representative from a methanogen known to catalyze this reaction (Jetten et al. 1991).

The K_m values of CODH/acetyl-CoA synthase for CO are in the range of 0.7–12 mM (Meyer & Fiebig 1985) and hence are 1 to 5 orders of magnitude higher than those of carboxidotrophic bacteria (Table 2). An exceptionally low K_m value of 10 μM was found with the partially purified enzyme from *D. desulfuricans* (Meyer & Fiebig 1985). The enzyme was shown to contain nickel and the UV/VIS spectrum showed similarities to CODH/acetyl-CoA synthase of acetogens. A similar low K_m

value (60 μM) was determined for CODH in cell extracts of *D. autotrophicum* (Schauder et al. 1989).

IV. Participation of microorganisms in the CO cycle

A. Sources of CO

Anthropogenic and natural emissions of CO were estimated to be 12–14 · 10^8 metric tons · $year^{-1}$ (Lyons et al. 1984) and with increasing industry and traffic the amount of CO production is increasing rapidly. Conrad (1988) reported recent data on the production of CO due to anthropogenic (16.4 ± 8 · 10^8), biospheric (1.92 ± 1.2 · 10^8), and chemical sources (15 ± 8 · 10^8) giving a total amount of 33.32 ± 17.20 · 10^8 metric tons of CO produced per year. A scheme of the CO sources and sinks is shown in Fig. 5.

Zavarzin & Nozhevnikova (1977) suggested that under anaerobic conditions, soils produce CO and that at the level of ground water the concentration may reach more than 6 $\mu l \cdot l^{-1}$. Seiler (1978) demonstrated that soil liberates small amounts of CO and that the rate of CO production increases when the soil temperature exceeds 40° C. CO liberation by different soils in different climatic areas varies, but in relatively humid soils of temperate regions, CO consumption is 5 to 10 times higher than the CO liberation (Conrad & Seiler 1985a,b).

CO is also produced by a number of living beings and occurs as a product during the degradation of the α-methine bridge in porphyrins by mammals, green and red algae, and cyanobacteria (Troxler 1972; Troxler & Dokos 1973; Yoshida et al. 1982) as well as during the degradation of flavonoides, quercitrin, and rutin by fungi (Westlake et al. 1959; Simpson et al. 1960, 1963). Marine animals of the order *Siphonophora* produce substantial amounts of CO in their pneumatophores (Hahn & Copeland 1966; Wittenberg 1960; Wittenberg et al. 1962; Pickwell et al. 1964; Pickwell 1970) and the brown alga *Nereocystis luetkeana* contains, in addition to other gases (e.g. O_2, N_2, CO_2), up to 12% (v/v) CO in its pneumatocysts (Chapman & Tocher 1966).

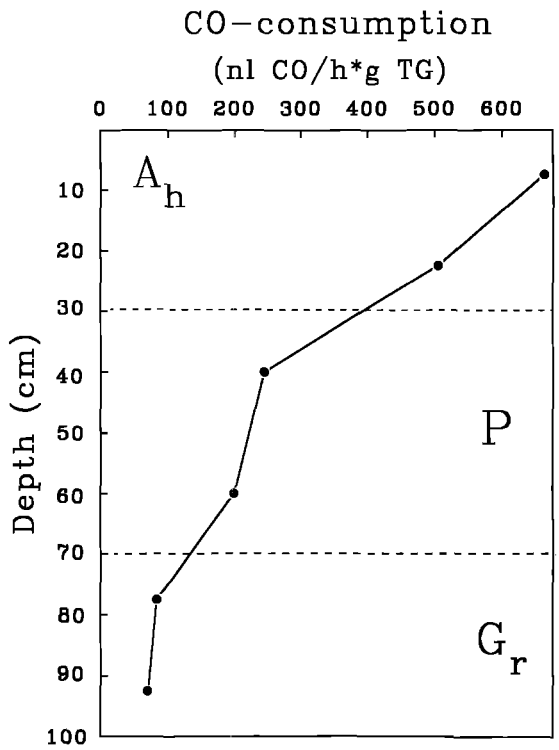

Fig. 6. CO consumption by soil samples collected at fallow land. A_h, P, and G_r are the soil horizons.

Seedlings of higher plants such as pea, cucumber, turnip, and rye liberate CO during germination and also during photorespiration, plants produce CO (Fischer & Lüttge 1978, 1979). The authors also observed that CO production was proportional to light intensity and that it was higher with C_3 plants than with C_4 plants.

B. Fate of CO in the environment

Soil is one of the major sinks for atmospheric CO. Conrad & Seiler (1980) reported that 5 · 10^8 metric tons CO · $year^{-1}$ were consumed by soil, which at that time amounted to 40% of the total CO production. In the following years the total production of CO increased 3-fold (see above). The total CO consumption by soil, however, did not increase, thus resulting in a decrease of the percentage of CO (15 %) consumed by soil (Conrad 1988). Nevertheless, CO is present in the atmosphere only as a

trace gas (0.06 to 0.15 ppm) suggesting that it might be rapidly converted to CO_2 or incorporated into organic material. The remaining amount of CO could be removed from the troposphere by chemical oxidation or by diffusion into the stratosphere (Fig. 5). In the northern hemisphere the concentration of CO is 0.15 ppm, whereas in the southern hemisphere it is only 0.06 ppm. This difference was ascribed to the greater extent of industrialization in the north (Seiler 1974, 1978; Heidt et al. 1980; Robinson et al. 1984). Although the CO content of the atmosphere is only in the trace range, the cycle of this gas is of vital importance for life on earth and has a key function in air chemistry, where it contributes to the climatic changes on earth (Conrad 1988).

C. Soil as the biological sink for CO

The consumption of CO by soil is a biological process. Sterilized soil does not show any CO consumption, but it resumes its CO-consuming activity after the addition of bacteria or nonsterile soil (Inman et al. 1971; Ingersoll et al. 1974; Liebl & Seiler 1976; Seiler 1978). Many different microorganisms are responsible for the removal of CO from soil (Inman et al. 1971; Bartholomew & Alexander 1979; Spratt & Hubbard 1981; Duggin & Cataldo 1985) and Seiler et al. (1977) reported that the CO-consuming activity is highest at the surface layer of soil, where maximum microbial activities occur and the supply of oxygen is sufficient. CO consumption is independent of the temperature of surface soil (Conrad & Seiler 1985a,b).

In our own experiments, we observed CO consumption in soil profiles up to a depth of 100 cm (Fig. 6). CO consumption was related to the soil water potential and in surface soil at -0.03 MPa, the rate of CO oxidation was 25 μl CO $\cdot$ h^{-1} $\cdot$ g d. wt.$^{-1}$ and was reduced to 6 μl CO $\cdot$ h^{-1} $\cdot$ g d. wt.$^{-1}$ at -1.4 MPa. From the results obtained with pure cultures and from soil experiments (see next section), it can be concluded that aerobic carboxidotrophic bacteria play a significant role in the rapid removal of CO from the atmosphere.

D. Carboxidotrophic bacteria as CO consumers in soil

Conrad et al. (1981) compared the K_m and V_{max} values for CO of many carboxidotrophic bacteria (K_m = 465–1,110 μl CO $\cdot$ l^{-1}) with the K_m values of soils for CO (K_m = 5–8 μl CO $\cdot$ l^{-1}). They found that the K_m values of carboxidotrophic bacteria are 50 to 200 times higher than those of soils. Hence they concluded that carboxidotrophic bacteria do not play an important role in the removal of CO from the atmosphere and suggested the presence of other CO-oxidizing organisms in soil with higher affinity for CO. In other reports, the K_m values for various soils were described to range from 14.9–40 μl CO $\cdot$ l^{-1} (Bartholomew & Alexander 1981; Spratt & Hubbard 1981, Duggin & Cataldo 1985). Accordingly it was postulated that the major CO oxidation observed in soil is due to CO-cooxidizing microflora (Bartholomew & Alexander 1979, 1981, 1982). A number of ammonia oxidizers are known to be responsible for the oxidation of CO to CO_2 (Jones & Morita 1983; Jones et al. 1984) and also methanotrophs oxidize CO to CO_2 (Hubley et al. 1974; Ferenci 1974; Ferenci et al. 1975; Stirling & Dalton 1979).

Recent studies performed in our group with various soils and pure cultures of carboxidotrophic bacteria showed that the K_m values of soil and carboxidotrophic bacteria were similar. The K_m values of various soils were in the range of 8–42 μl CO $\cdot$ g d.wt.$^{-1}$ and the K_m values of various mesophilic and thermophilic carboxidotrophic bacteria were in the range of 64–130 μl CO $\cdot$ g d. wt.$^{-1}$ (Schricker et al. in preparation). These results indicate that carboxidotrophic bacteria probably participate in the oxidation of CO to a greater extent than it was believed before. It was also found that the rate of CO oxidation in acid soils was greater than the CO oxidation rates determined in neutral or alkaline soils.

Although a proper answer for the ecological importance or relevance of carboxidotrophic bacteria is not yet known, they are most probably scavengers of CO at micro- and macro-sites where the CO concentration increases to a level which might

be harmful to the other microflora. Even in the presence of higher concentrations of CO, some carboxidotrophic bacteria are able to utilize nitrate as electron acceptor during anaerobic respiration (Frunzke & Meyer 1990). Under natural conditions in anaerobic environments, where higher concentrations of CO and nitrate occur, CO-tolerant carboxidotrophic bacteria might be able to gain energy by nitrate respiration, which yields more energy than fermentation under anaerobic conditions.

S. thermoautotrophicus, a new carboxidotrophic bacterium, shows a unique gazotrophic pattern of metabolism, since it utilizes almost all main components (O_2, N_2, CO_2, H_2, CO) of air. In nature, where organic substrates and combined nitrogen are limiting microbial growth, bacteria belonging to this metabolic type may play an important role in removing CO from the air and such environments may be suitable for them to live without competition for nutrients.

V. Conclusions and perspectives

The utilization of CO by different metabolic groups of bacteria is now quite well understood at the physiological level. Also, the enzymes responsible for CO utilization are under investigation in carboxidotrophic, phototrophic, methanogenic, acetogenic, and sulfate-reducing bacteria.

The use of molecular biology methods will deepen the understanding of different CODH enzymes with respect to their structure, function, and the requirements for the regulation of their formation and assembly into active forms. Furthermore, the primary structure of CODH from *Rhodospirillum rubrum* should be determined, in order to see whether it is related to one of the other CO-oxidizing enzymes.

Although we know that soil is the biological sink for CO, we know almost nothing about the contributions of different metabolic groups of microorganisms to this sink. Information on capacities and limitations of the biological sink for CO is important in order to keep it active and to protect it against damage.

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Note added in proof

After completion of the manuscript, the nucleotide sequence of the genes encoding the 62 and 22 kDa proteins of *Rhodospirillum rubrum* CODH was published (Kerby et al. (1992) J. Bacteriol. 174: 5284–5294). The deduced amino acid sequence of the 62 kDa subunit shows similarity with the β subunit of *Clostridium thermoaceticum* (67%) and the α subunit of *Methanothrix soehngenii* (47%) CODH/acetyl-CoA synthase.

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