**CO Metabolism by a Thermophilic Actinomycete, *Streptomyces* Strain G26**

By JUDITH M. BELL,¹* CHRISTINE FALCONER,² JOHN COLBY¹ AND EDWIN WILLIAMS²

¹North East Biotechnology Centre, Biology Department, Sunderland Polytechnic, Sunderland SR1 3SD, UK.
²Microbiology Technology Group, The Medical School, The University, Newcastle-upon-Tyne NE1 7RU, UK.

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*Streptomyces* G26, isolated from compost, is a moderately thermophilic actinomycete and a facultative autotroph capable of aerobic growth on CO or on CO₂/H₂. Soluble extracts of CO-grown mycelium contained enzymes of the Calvin cycle together with a CO oxidoreductase; the latter was present in heterotrophically grown mycelium at only 1% of the level found in CO-grown cultures. The CO oxidoreductase of *Streptomyces* G26 was unique in its ability to rapidly reduce low-potential acceptors such as the viologen dyes in addition to acceptors with $E_0$ values between $+11$ mV and $+429$ mV. Although complete purification of the enzyme proved difficult, evidence is presented that extracts contained only one CO oxidoreductase. The partially purified enzyme had a very low apparent $K_m$ for CO, comparable with the CO oxidoreductase from *Pseudomonas thermocarboxydovorans*. Highest activities were observed at pH 7.2 and 60-65 °C. The enzyme was inactivated by methanol, suggesting that it is a molybdenum hydroxylase like the CO oxidoreductases from other aerobic bacteria.

**INTRODUCTION**

The ability of several species of *Pseudomonas*, *Azomonas* and other aerobic Gram-negative bacteria to oxidize CO to CO₂ is due to the presence in these bacteria of a CO oxidoreductase (CO oxidase; CO: acceptor oxidoreductase; CO dehydrogenase; EC 1.2.99.2) (Williams et al., 1986). During CO-autotrophic growth, CO is first oxidized to CO₂ and the energy from this oxidation is harnessed to drive CO₂ uptake via the Calvin cycle. For *Pseudomonas thermocarboxydovorans*, the stoichiometry of this reaction is as follows (Williams et al., 1987), showing that 16% of the CO carbon is assimilated into cell carbon.

$$1 \text{ g CO} + 0.357 \text{ g O}_2 \rightarrow 1.310 \text{ g CO}_2 + 0.097 \text{ g cells}$$

The proportion of carbon fixed varies from species to species, and with growth conditions, with from 2% to 16% of the CO₂ arising from CO oxidation being assimilated into cell carbon (Meyer & Schlegel, 1983).

The utilization of CO by Gram-positive bacteria has not been extensively studied. Several actinomycetes oxidize CO to CO₂ during growth on complex media (Bartholomew & Alexander, 1979), although no investigation into the physiological mechanism of this activity has been published. Meyer & Schlegel (1983) described two non-mycelial strains, *Arthrobacter* 11x and *Bacillus schlegelii*, that grow well on CO as sole source of carbon and energy; both organisms were originally isolated as hydrogen bacteria. Bell et al. (1985) described a thermophilic, mycelial carboxydotroph, *Streptomyces* G26, that grows rapidly on CO; we have also reported

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*Abbreviations:* BV, benzyl viologen; pCMB, p-chloromercuribenzoate; DCPIP, 2,6-dichlorophenolindophenol; MB, methylene blue; MV, methyl viologen; PES, phenazine ethosulphate; PMS, phenazine methosulphate.
the isolation and characterization of three other strains of thermophilic, CO-utilizing actinomycetes (Williams et al., 1987).

The physiology of carboxydrotrophic bacteria, and their potential importance of biotechnology, has been discussed in several recent reviews (Colby et al., 1985; Meyer et al., 1986, Williams et al., 1986, 1987). As part of a programme to study CO metabolism in thermophilic carboxydrotrophs, we have investigated the properties of the CO oxidoreductase from Streptomyces G26. This organism was chosen to represent more than 100 strains of CO-utilizing actinomycetes that we have isolated from soil and compost.

METHODS

Growth medium and conditions. Streptomyces G26 was grown autotrophically in mineral base E (Owens & Keddie, 1969), incubated at 50 °C under an atmosphere containing CO as sole carbon and energy source as described by Lyons et al. (1984). The medium was supplemented with p-amino benzoic acid (10 μg ml⁻¹). Heterotrophic cultures were grown on mineral base E supplemented with 0.2% sodium pyruvate, inositol or glucose. Stock cultures were maintained on mineral base E solidified with 1.5% (v/v) Oxoid purified agar incubated under 30% (v/v) CO in air or on Bennett's medium (Jones, 1949). For long-term storage, cultures were maintained as freeze-dried suspensions containing 5% (w/v) skimmed milk as cryoprotectant. Cultures could also be maintained at liquid nitrogen temperatures as a buffered cell suspension containing 10% (v/v) glycerol.

Bulk growth of Streptomyces G26. Cultures were grown on CO in 3 l batches in a magnetically driven fermenter as described by Lyons et al. (1984). The fermenter, containing 3 l of medium prewarmed to 50 °C, was inoculated with 300 ml of a 3-d-old shake flask culture, grown under an atmosphere containing 25% (v/v) CO in air. Similar methods were used to grow heterotrophic cultures on 0.4% sodium pyruvate, glucose or inositol. When the A₅₄₀ of the culture had risen to about 2, approximately 2.5 l of the culture was drawn off and the remainder left to act as an inoculum for the following batch. The harvested bacterial suspension was centrifuged at 12000 g for 10 min at 4 °C, washed with ice-cold 50 mM-sodium phosphate buffer, pH 7.0, and then the bacterial pellets were stored at −20 °C until required. Mycelial extracts (crude soluble extract) were prepared by sonication as described by Lyons et al. (1984) or by a single passage through a French press at 137 MPa in the presence of 5 mM-MgCl₂ and 0.3 mg DNAase ml⁻¹.

Protein estimation. The concentration of protein in crude soluble extracts, and in extracts partially purified by anion exchange chromatography, were determined using the method of Lowry, as modified by Kennedy & Fewson (1968) using crystalline bovine serum albumin (fraction V) as standard.

Standard spectrophotometric assay. The assay used was that described by Turner et al. (1984). One unit of CO oxidoreductase activity is defined as the amount of enzyme catalysing the reduction of 1 μmol DCPIP min⁻¹. The millimolar extinction coefficients of DCPIP at 600 nm were taken to be 20.65 cm⁻¹ at pH 7.0 and 21.08 cm⁻¹ at pH 7.2 (Armstrong, 1964). Standard oxygen electrode assay. A Clark-type oxygen electrode (Rank) was used to measure CO oxidoreductase activity with auto-oxidizable electron acceptors. Reaction mixtures (3 ml) contained 0.15 mmol Tris/HCl buffer, pH 7.0; 1.5 μmol PES and 1.0 ml CO-saturated glass-distilled water. The reaction was started by the addition of enzyme solution, by syringe, through the port in the cap. The oxygen electrode output was calibrated by saturating the contents of the reaction chamber with gas mixtures of known partial pressure of oxygen. The solubility of oxygen in water at 50 °C was taken to be 24.6 μl ml⁻¹ (Weast, 1979). One unit of CO oxidoreductase activity is defined as the amount of enzyme catalysing the uptake of 1 μmol O₂ min⁻¹.

Gas chromatographic determination of CO. This was done as described by Lyons et al. (1984).

Preparation of partially purified extracts by anion-exchange chromatography. All procedures were done at 4 °C. Crude soluble extract containing about 500 mg protein was pumped onto a 35 × 2.5 cm column of DEAE-Sephacel (Pharmacia), and the column was washed with 20 mm-Tris/HCl buffer, pH 7.0, at a flow rate of 1 ml min⁻¹ until material absorbing at 280 nm ceased to elute from the column. A salt gradient (0.0–0.6 M-NaCl in 20 mm-Tris/HCl buffer, pH 7.0) was used to elute the components remaining attached to the column matrix. Fractions containing CO oxidoreductase activity were located using the standard spectrophotometric assay and were then pooled, drop-frozen in liquid nitrogen and stored at −20 °C until required.

Chemicals. DNAase from bovine pancreas, DCPIP, PES, PMS, FAD, FMN, nitro blue tetrazolium, toluidine blue O, BV, MV, yeast NAD and NADP, horse heart cytochrome c, 2,2'-dipyridyl, iodoacetate, N-ethylmaleimide, 5,5'-dithiobis(2-nitrobenzoic acid), pCMB, semicarbazide and hydroxylamine were obtained from Sigma. Thionine (Gurr), brilliant cresyl blue (Gurr), MB, neutral red, potassium ferricyanide, 8-hydroxyquinoline, sodium azide, Na, EDTA, 2,9-dimethyl-1,10-phenanthroline (neocuproine), sodium chlorate, NaF and KCN were supplied by BDH.
RESULTS

Growth of Streptomyces G26 under autotrophic and heterotrophic conditions

Streptomyces G26 grows autotrophically in mineral base E with CO (25–35%, v/v, in air) as sole carbon and energy source, and also as a hydrogen bacterium on the same medium under an atmosphere containing (by vol.) 45% H₂, 5% CO₂ and 50% air. Complex media such as nutrient agar, Bennett's medium and Kuster and William's medium (Kuster & Williams, 1964) also support growth. Some growth occurred on all of these media, but was fastest on Bennett's medium. Growth on Bennett's medium, determined over a 6 d incubation period, occurred between 45 and 60 °C and fastest growth occurred at 50 °C. The temperature range for autotrophic growth was investigated using mineral base E incubated under 25% (v/v) CO. Growth as a waxy pellicle was just perceptible after 6 d incubation at 37 °C, but otherwise followed the pattern achieved on complex medium with fastest growth observed at 50 °C.

The filamentous nature of the organism made the measurement of its growth rate difficult. When grown in mineral base E on CO under static conditions, the culture formed a white, waxy pellicle that did not pellet on centrifugation at 6000 g for 10 min. When incubated with shaking at 200 r.p.m., growth occurred as discrete, visible filaments of variable size that were submerged in the medium. These filaments could be harvested by centrifugation. When cultured in shake flasks on complex medium, growth occurred as round pellets up to 3 mm in diameter. Stock cultures repeatedly subcultured on Bennett's medium for a period of 12 months still retained the ability to grow on CO.

For biochemical studies, Streptomyces G26 was grown on CO by a batch process, using a fermenter of 3 l working volume. At an A₅₄₀ of 0.1, the culture appeared homogeneous and absorbance readings were constant, sample to sample. At lower cell densities, the culture appeared to consist of discrete filaments, giving slightly variable absorbance readings. Cultures were harvested at an A₅₄₀ of 2, midway through the linear growth phase; exponential growth was not observed. Negatively stained electron micrographs, prepared directly from drops of a culture sample taken immediately before harvesting, showed Streptomyces G26 to grow as loose branching filaments under these conditions (Fig. 1). Individual cells observed were 0.4 μm wide and varied greatly in length; cells up to 10 μm long were frequently observed. Samples of Streptomyces G26 were also embedded in resin and cell-sections prepared. Fig. 2 shows a typical section through a filament showing cell wall formation at a mycelial branch-point. No special organelles or membrane structures, which might be associated with CO metabolism, were observed.

Enzyme activities in crude cell-extracts

Many of the important enzymes of the Calvin cycle, and those associated with other C₁-assimilation pathways or with the recovery of phosphoglycollate, were assayed using crude soluble extracts of mycelium grown with CO or with sodium pyruvate as carbon and energy source (Table 1). The Calvin cycle enzymes were present in sufficiently high activity to account for the growth rate on CO, and in most cases enzyme synthesis was clearly induced during growth on CO compared with heterotrophic growth on pyruvate. Hexulose phosphate synthase and hydroxypyruvate reductase, key enzymes of the ribulose monophosphate and serine pathways for C₁-assimilation respectively, were absent. Phosphoglycollate phosphatase was present in extracts of both autotrophically grown and heterotrophically grown mycelium.

Demonstration of CO oxidoreductase activity in crude soluble extracts and the preparation of partially purified enzyme

Washed mycelial suspensions were broken by ultrasonication or by using a French pressure cell: CO oxidoreductase activities in the soluble extracts were 1.73 U mg⁻¹ and 0.81 U mg⁻¹ respectively. All subsequent work was done with sonicated preparations. The crude soluble extract prepared from Streptomyces G26 differed from that prepared by the same method from P. thermocarboxydovorans (Lyons et al., 1984) in that it did not contain components which catalyse the re-oxidation of reduced DCPIP under the standard assay conditions. Strictly anaerobic conditions were therefore unnecessary when assaying crude preparations.
CO oxidoreductase was inducible. When prepared from mycelium that had been grown on Bennett's medium, or mineral base E plus glucose or inositol, crude soluble extracts contained CO oxidoreductase activities of about 0.02 U (mg protein)$^{-1}$, representing only about 1% of the specific activity in CO-grown cells.

CO oxidoreductase was partially purified from crude soluble extracts of *Streptomyces* G26 by anion-exchange chromatography on DEAE-cellulose as described in Methods. Activity eluted at 0.33 M-NaCl as a single sharp peak with a specific activity about 3-fold greater than the crude extract and a yield of 46%. The methods used for the complete purification of CO oxidoreductase from *P. carboxydovorans* (Meyer & Schlegel, 1980) and from *P. thermocarboxydovorans* (Turner *et al.*, 1984; A. Halder, personal communication) were not successful with this enzyme and attempts at further purification resulted in irreversible loss of enzyme activity.

Fig. 1. Electron micrograph of *Streptomyces* G26 mycelium. A sample of mycelium growing autotrophically on CO in the fermenter was harvested, washed and negatively stained with 1% silicotungstate. Bar, 4 μm.
Fig. 2. Electron micrograph of a cell section of *Streptomyces G26* mycelium. Washed mycelium was fixed in 2.5% (v/v) glutaraldehyde followed by 1% (w/v) osmium tetroxide and 1% (v/v) aqueous uranyl acetate. Bar, 0.25 μm.

Properties of partially purified CO oxidoreductase

Using either the standard spectrophotometric or the standard oxygen electrode assay, a linear relationship was observed between measured enzyme activity and enzyme concentration over the range 25–100 μg protein.

Reduction of electron acceptors. CO oxidoreductase activity with a range of auto-oxidizable electron acceptors was determined using the standard oxygen electrode assay; other acceptors
Table 1. Activity of autotrophic enzymes in crude sonic extracts of CO- and pyruvate-grown mycelium

Crude soluble extracts were prepared as described in Methods. Enzyme assays were done as described by Lyons et al. (1984).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity [mU (mg protein)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO-grown bacteria</td>
</tr>
<tr>
<td>Ribulose-1,5-bisphosphate carboxylase</td>
<td>61</td>
</tr>
<tr>
<td>Fructose-1,6-diphosphatase</td>
<td>163.4</td>
</tr>
<tr>
<td>Sedoheptulose-1,7-diphosphatase</td>
<td>76.4</td>
</tr>
<tr>
<td>Transaldolase</td>
<td>118.3</td>
</tr>
<tr>
<td>Transketolase</td>
<td>93.0</td>
</tr>
<tr>
<td>Hydroxypyruvate reductase</td>
<td>0</td>
</tr>
<tr>
<td>Phosphoglycolate phosphatase</td>
<td>79.0</td>
</tr>
<tr>
<td>Hexulose phosphate synthase</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Table 2. Reduction of various electron acceptors by partially purified CO oxidase

Enzyme activities with non-auto-oxidizable acceptors (NAD, NADP, DCPIP, cytochrome c, potassium ferricyanide) were measured in a modified spectrophotometric assay in which the acceptor, at the concentration shown in the table, replaced PES. The millimolar extinction coefficients used to calculate the activities were cytochrome c (19-7), NADH (6-22), NADPH (6-0), and potassium ferricyanide (1-0) (cm⁻¹). Other acceptors were tested in the oxygen electrode assay. The rate with PES (100%) was 5.0 U (mg protein⁻¹).

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>E₀ (mV)</th>
<th>Conc (mm)</th>
<th>Reduction rate (percentage of rate with PES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>+816</td>
<td>1.20</td>
<td>0</td>
</tr>
<tr>
<td>Potassium ferricyanide</td>
<td>+429</td>
<td>1.00</td>
<td>66</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>+245</td>
<td>0.10</td>
<td>10</td>
</tr>
<tr>
<td>DCPIP</td>
<td>+217</td>
<td>0.09</td>
<td>27</td>
</tr>
<tr>
<td>Toluidine blue O</td>
<td>+110</td>
<td>0.05</td>
<td>66</td>
</tr>
<tr>
<td>PMS</td>
<td>+80</td>
<td>0.05</td>
<td>120</td>
</tr>
<tr>
<td>Thionine</td>
<td>+70</td>
<td>0.05</td>
<td>74</td>
</tr>
<tr>
<td>PES</td>
<td>+55</td>
<td>0.05</td>
<td>100</td>
</tr>
<tr>
<td>Brilliant cresyl blue</td>
<td>+47</td>
<td>0.05</td>
<td>79</td>
</tr>
<tr>
<td>MB</td>
<td>+11</td>
<td>0.05</td>
<td>73</td>
</tr>
<tr>
<td>FAD/FMN</td>
<td>-219</td>
<td>1.00</td>
<td>0</td>
</tr>
<tr>
<td>Neutral red</td>
<td>-320</td>
<td>0.05</td>
<td>11</td>
</tr>
<tr>
<td>NAD (NADP)</td>
<td>-320(4)</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>BV</td>
<td>-359</td>
<td>0.05</td>
<td>117</td>
</tr>
<tr>
<td>MV</td>
<td>-440</td>
<td>0.05</td>
<td>30</td>
</tr>
</tbody>
</table>

were tested in a modified spectrophotometric assay. There was no direct correlation between standard redox potential (E₀) and the rate of reduction by CO oxidoreductase (Table 2). The highest rates of reduction were found with acceptors of about +11 mV to +429 mV redox potential but also with viologen dyes (E₀ = -359 mV and -440 mV). No interaction with cytoplasmic acceptors such as nicotinamide nucleotides or flavins was observed.

Uptake of CO during catalysis. This experiment confirmed that the reduction of artificial electron acceptors of both high (PES) and low (BV) redox potential is linked to the uptake of CO. CO uptake was measured by determining the CO concentration in the gas phase of a sealed assay (Fig. 3). The concentration of CO in the samples containing either electron acceptor appeared to fall at approximately the same rate. The CO concentration in the control assay without partially purified extract remained constant, but some reduction was seen in assays containing no electron acceptor. This may be explained by the presence of a natural electron acceptor in the enzyme preparation used.
Activity of CO oxidoreductase under anaerobic conditions. Further confirmation of the ability of CO oxidoreductase to catalyse the reduction of the viologen dyes was obtained by direct measurement of the formation of the reduced forms of the dyes in an anaerobic spectrophotometric assay based on that of Meyer & Schlegel (1980). Activity with MB [2.42 U (mg protein)] was about double that observed with MV and BV [1.40 and 1.13 U (mg protein)] respectively. Reduction of MB was linear starting immediately on addition of enzyme solution to the assay cuvette. In contrast, the reduction of viologen dyes began after a short lag, probably a consequence of re-oxidation of the viologen dyes by residual oxygen. When calculating the relative activities, the stoichiometry of CO oxidation with the different electron acceptors was taken into account. MB is reduced by CO in a 1:1 ratio whilst viologens are reduced by CO at a ratio of 2:1 (Diekert & Thauer, 1978). An assay prepared with the CO-flushing step omitted did not support the reduction of any of the electron acceptors. Some activity, however, was restored by gentle addition of 0.5 ml CO gas to the assay cuvette.

Comparison of CO:PES and CO:BV oxidoreductase activities of partially purified extract

The unexpected finding that partially purified extracts of Streptomyces G26 catalysed the reduction of both PES ($E'_0 = +55$ mV) and BV ($E'_0 = -359$ mV) with CO might be explained by the presence of two enzymes, each catalysing the reduction of one of these acceptors, or of a single enzyme of low electron acceptor specificity. A series of experiments was devised to clarify this as follows.

Effect of pH. In a preliminary experiment, the activity of CO oxidoreductase using PES as electron acceptor was determined over the range pH 5.0–9.0 using the standard spectrophotometric assay. Buffers (100 mM) were: MES/NaOH (pH 5.0–7.0), sodium phosphate (pH 6.0–8.0), tricine/HCl (pH 7.0–9.0), Tris/HCl (pH 7.0–9.0) and glycine/NaOH (pH 8.0–10.0). The use of different buffers appeared to affect enzyme activity but, in general, more than 50% maximum activity occurred over the range pH 6.0–8.5. CO oxidoreductase activity with PES and BV as electron acceptors was compared over the range pH 6.0–8.0 using sodium phosphate buffer. CO:BV oxidoreductase activity was determined by using the standard oxygen electrode assay. Identical pH profiles were observed with both acceptors and maximum activity was at pH 7.2. In calculating the results, the relationship between the molar extinction coefficient for DCPIP and pH (Armstrong, 1964) was taken into account.
Fig. 4. Temperature stability of CO: PES (a) and CO: BV (b) oxidoreductase activities. Samples (0.5 ml) of partially purified extract were placed in three microfuge tubes at 50 (■), 55 (□) or 60 °C (∆). Samples were taken at intervals and enzyme activities with PES and BV were determined using the standard spectrophotometric assay and the oxygen electrode assay respectively. Initial activities with PES and BV were 5.0 and 5.7 U (mg protein)^{-1} respectively.

Fig. 5. Effect of temperature on CO: PES (∆) and CO: BV (□) oxidoreductase activities, determined using the standard spectrophotometric assay and the oxygen electrode assay respectively.

**Temperature stability.** The stability of CO oxidoreductase as measured with PES or BV as oxidizing substrate, was determined at 50, 55 and 60 °C. Inactivation curves (Fig. 4) indicate that the stability of CO: PES oxidoreductase and CO: BV oxidoreductase activities were essentially the same at the temperatures investigated. Activity halved within the first 9 min of incubation at 50 °C and within 4 min at 55 °C. Almost all activity was lost during incubation at 60 °C for 5 min. Temperature/activity curves obtained with particular enzyme preparations were identical with PES or BV (Fig. 5), although the actual temperature at which maximum activity was observed varied over the range 60–65 °C depending on the preparation and protein concentration used.

**Effect of enzyme inhibitors.** The effect of a variety of known enzyme inactivators on the activity of CO oxidoreductase, measured with PES or BV as primary electron acceptor, was investigated. The range of inactivating compounds and their concentrations were chosen for comparison with similar experiments done on the CO oxidoreductase from *P. thermocarboxydovorans* in this laboratory (Bell et al., 1985). Most of the chelating reagents tested (1 mM-8-hydroxyquinoline; 10 mM-sodium azide; 10 mM-EDTA; 1 mM-2,9-dimethyl-1,10-phenanthro-
Fig. 6. Effect of methanol (a) and ethanol (b) on CO oxidoreductase activity. Enzyme activity was determined using the standard spectrophotometric assay with PES as electron acceptor. Reactions were started by the addition of partially purified extract to reaction mixtures containing methanol or ethanol (■, 20 mM; ▲, 80 mM; ○, 400 mM; □, 800 mM). □, Control (no methanol or ethanol).

line; 10 mM-sodium chlorate; 10 mM-NaF; 10 mM-2,2'-dipyridyl; 0·1-10 mM-KCN) had very little or no effect on CO oxidoreductase activity with either electron acceptor; however, 10 mM-2,2'-dipyridyl reduced the activity by 35-39% (depending on whether PES or BV was used) and cyanide was strongly inhibitory, 65% of CO oxidoreductase activity being lost at 1 mM concentration.

Of the thiol-binding reagents, 10 mM-N-ethylmaleimide, 5,5'-dithiobis(2-nitrobenzoic acid) and iodoacetate had no significant effect on either CO:PES or CO:BV oxidoreductase activity, whereas both activities were completely abolished when the enzyme was pre-incubated with 2 mM-pCMB; 50 μM-pCMB reduced measurable activity by 12%, showing it to be the most effective inhibitor of those tested. Pre-incubation of the CO oxidoreductase preparation with the carbonyl reagents semicarbazide or hydroxylamine (both at 10 mM) had no significant inhibitory effect on CO oxidoreductase activity with either PES or BV as electron acceptor.

In all cases, the sensitivity to each inhibitor was essentially the same for both the CO:PES oxidoreductase and the CO:BV oxidoreductase activities.

Other characteristics of Streptomyces G26 CO oxidoreductase

$K_m$ for electron acceptors and for CO. CO oxidoreductase activity with a wide range of acceptor concentrations was determined by using the standard oxygen electrode assay at pH 7·0. From Lineweaver–Burk double reciprocal plots, the apparent $K_m$ values for PES, BV and MV were calculated to be 2·94 μM, 0·18 mM and 2·27 mM respectively. An apparent $K_m$ value for CO of 1·75 μM was determined by using the spectrophotometric assay. In each case the $K_m$ value quoted is the mean of three determinations made with different enzyme preparations.

Effect of methanol on activity. The effect of methanol on CO oxidoreductase activity, measured by using the standard spectrophotometric assay, is shown in Fig. 6(a). Enzyme activity appeared to decrease with incubation time, but this may be accounted for by heat-inactivation of the enzyme at 50 °C (see Fig. 4). A methanol concentration of 0·8 M was required to almost abolish enzyme activity. Methanol inactivation was turnover-dependent. A sample of enzyme solution incubated in the presence of 0·8 M-methanol and the absence of substrate for 2 min at 50 °C showed no loss of CO oxidizing activity, as measured by following PES reduction, relative to a control in which the reaction was started by adding the enzyme. Ethanol was also inhibitory, but to a lesser degree (Fig. 6b). The results obtained for methanol and ethanol inactivation of CO oxidoreductase from Streptomyces G26 were very similar to those obtained with the CO oxidoreductase from P. thermocarboxydovorans (A. Halder, personal communication).
**DISCUSSION**

*Streptomyces* G26 can grow on CO as sole carbon and energy source and it might be expected, therefore, that this organism would possess a CO oxidoreductase similar to that found in the Gram-negative aerobic carboxydotrophic bacteria. These CO oxidoreductases are complex oligomeric enzymes containing bactopterin (Meyer & Rajagopalan, 1984; Krüger & Meyer, 1986), FAD and two types of iron–sulphur centres (Meyer, 1982; Bray et al., 1983).

Crude soluble extracts of *Streptomyces* G26 were shown to catalyse the reduction of a number of artificial electron acceptors on addition of CO to the assay system. This suggests the presence of an enzyme in the soluble cell fraction catalysing the following reaction

\[ \text{CO} + H_2O + \text{acceptor}_{\text{ox}} \rightarrow \text{CO}_2 + \text{acceptor}_{\text{red}} \]

where ‘acceptor’ can be any one of the following: PES, PMS, DCPIP, MB, BV, MV, toluidine blue O, thionine, brilliant cresyl blue, neutral red, horse heart cytochrome c or potassium ferricyanide. This ability to interact with the viologen dyes was surprising as the CO-dependent reduction of these electron acceptors had not been demonstrated in CO oxidoreductases from other aerobic bacteria (Bell et al., 1985), although a slow CO-dependent reduction of MV and BV has been observed with crude extracts of the thermophilic Gram-positive carboxydotroph *Bacillus schlegelii* (Krüger & Meyer, 1984).

CO-dependent reduction of MV is well-documented for CO dehydrogenases from anaerobic bacteria (Diekert & Thauer, 1978; Drake et al., 1980). CO dehydrogenases from anaerobes contain nickel and are oxygen-sensitive. Although these two classes of enzymes formally catalyse the same reaction, they are completely different in molecular and kinetic properties and indeed in cellular function, and the use of the terms ‘CO oxidoreductase’ and ‘CO dehydrogenase’ is a useful shorthand to distinguish between them.

Two classes of electron acceptor were active: (i) acceptors with \( E_0 \) values between +11 and +429 mV that are effective with other CO oxidoreductases; (ii) the viologen dyes with \( E_0 \) values between −329 and −440 mV. The latter are inactive with CO oxidoreductases but are active with CO dehydrogenases. This suggests that either (i) a single CO oxidoreductase activity is present that has a very low specificity towards oxidizing substrate, or (ii) a CO oxidoreductase with a similar redox range to the isofunctional enzyme from other carboxydotrophs is present, together with a CO oxidizing enzyme able to interact with low-potential electron acceptors. If two separate enzymes were present, it might be expected that these enzymes would differ in some measurable parameters, e.g. heat stability, pH optimum, temperature optimum or inhibitor sensitivity. These parameters were investigated for the CO-dependent reduction of PES and BV by partially purified extract of *Streptomyces* G26. In no case was any significant difference between the two activities observed, suggesting that only one enzyme activity is present.

The \( K_m \) for CO for the *Streptomyces* G26 CO oxidoreductase, at 1.75 μM, is similar to that found for *P. thermocarboxydovorans* CO oxidoreductase, at 0.5 μM (Turner et al., 1984). Lower affinities were found with the CO oxidoreductases from mesophilic carboxydobacteria, e.g. *P. carboxydovorans* at 53 μM (Meyer & Schlegel, 1980) and *P. carboxydohydrogena* at 63 μM (Kim & Hegeman, 1981).

The CO oxidoreductases from *P. carboxydovorans* and *P. thermocarboxydovorans* are molybdenum hydroxylases and, like xanthine oxidase and aldehyde oxidase, contain Mo, FAD, acid-labile sulphide and Fe in the ratio 1:1:4:4 (Meyer, 1982; Turner et al., 1984). A unique property of molybdenum hydroxylases is their susceptibility to inactivation by methanol (Rajagopalan & Handler, 1964, 1968). *Streptomyces* G26 CO oxidoreductase was also inactivated by methanol and its susceptibility was comparable with the xanthine dehydrogenase of *Veillonella alcalescens* (formerly *Micrococcus lactilyticus*) (98% inhibited by 0.5 M-methanol), as investigated by Smith et al. (1967). This is in contrast to the CO oxidoreductase from *P. carboxydovorans*, which was almost completely inhibited by 0.125 M-methanol (Meyer, 1982). Milk xanthine oxidase shows much higher resistance to methanol and was inhibited only 15% by 0.5 M-methanol (Smith et al., 1967). This variation in susceptibility may be dependent on active site structure.
Methanol inactivation of V. alcalescens and chicken liver xanthine dehydrogenases, P. carboxydovorans and rabbit liver aldehyde oxidase was found to be progressive and turnover-dependent; no inactivation was observed in the absence of reducing substrate (Dalton et al., 1976; Meyer, 1982; Rajagopalan & Handler, 1967; Smith et al., 1967). Inactivation of Streptomyces G26 CO oxidoreductase was apparently non-progressive as inactivation over the 5 min incubation period was no more marked in assays containing methanol than in a control assay with no added methanol, where the reduction in activity may be explained by heat-inactivation of the enzyme. Methanol inactivation was, however, turnover-dependent. Similar behaviour has been observed for the CO oxidoreductase from P. thermocarboxydovorans, where methanol was shown to be a non-competitive inhibitor (A. Halder, personal communication). Inhibition of nitrate reductase by methanol was described as immediate and constant i.e. non-progressive (Rajagopalan & Handler, 1964). However, unlike Streptomyces G26 CO oxidoreductase and other molybdenum hydroxylases investigated, ethanol was a more effective inhibitor than methanol: 1 M-methanol reduced activity by 32% compared to 1 M-ethanol at 44%.

Susceptibility to inactivation by methanol suggests that the Streptomyces G26 CO oxidoreductase is a molybdenum hydroxylase, like the isofunctional enzymes from P. carboxydovorans and P. thermocarboxydovorans. Confirmation of this must await its complete purification and characterization. If indeed confirmed, this still leaves unexplained the ability of the enzyme to use viologens. CO dehydrogenases contain 4Fe-4S centres rather than the 2Fe-2S centres found in the CO oxidoreductases studied so far (Ragsdale et al., 1982; Bray et al., 1983).

The presence of phosphoglycollate phosphatase activity in extracts may indicate that Streptomyces G26, like P. thermocarboxydovorans (Lyons et al., 1984), has a mechanism for the assimilation of phosphoglycollate arising from the oxygenase activity of D-ribulose-1,5-bisphosphate carboxylase. The glycine-serine pathway, which probably occurs in Streptomyces G26 during growth on CO, cannot be discounted at this stage.

The susceptibility to inactivation of the enzyme to use viologens. CO dehydrogenases contain 4Fe-4S centres rather than the 2Fe-2S centres found in the CO oxidoreductases studied so far (Ragsdale et al., 1982; Bray et al., 1983). The possibility that Streptomyces G26 CO oxidoreductase also contains such 4Fe-4S centres cannot be discounted at this stage.

The presence of phosphoglycollate phosphatase activity in extracts may indicate that Streptomyces G26, like P. thermocarboxydovorans (Lyons et al., 1984), has a mechanism for the assimilation of phosphoglycollate arising from the oxygenase activity of D-ribulose-1,5-bisphosphate carboxylase. The glycine-serine pathway, which probably occurs in P. thermocarboxydovorans, may not occur in Streptomyces G26 as no hydroxypyruvate reductase activity was found. Other pathways for glycollate metabolism in photoautotrophs and chemoheterotrophs have been discussed by Beudeker et al., (1981). Further work is necessary to establish which pathway occurs in Streptomyces G26 during growth on CO.

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