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Carbon Monoxide Dehydrogenase in Cell Extracts of an *Acinetobacter* Isolate

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Acinetobacter sp. 1의 일산화탄소 산화효소의 특성

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Abstract: Extracts of CO-autotrophically grown cells of *Acinetobacter* sp. 1 were shown to use thionin, methylene blue, or 2,6-dichlorophenol-indophenol, but not NAD, NADP, FAD, or FMN, as electron acceptors for the oxidation of CO under strictly anaerobic conditions. The CO dehydrogenase (CO-DH) in the this bacterium was found to be an inducible enzyme. The enzyme activity was determined by an assay based on the CO-dependent reduction of thionin. Maximal reaction rates were found at pH 7.5 and 60°C, and the Arrhenius plot revealed an activation energy of 6.1 kcal/mol (25.5 kJ/mol). The K_m for CO was 154 μM. Known metal chelating agents tested had no effects on the CO-DH activity. No divalent cations tested affect the enzyme activity significantly except Cu^{2+} which suppressed the activity completely. The enzyme was inhibited by glucose and succinate. The same extracts catalyzed oxidation of hydrogen gas and formate with thionin as electron acceptor. The CO-DH of *Acinetobacter* sp. 1 was found to have no immunological relationship with that of *Pseudomonas carboxydohydrogena.*

Kew Words: CO dehydrogenase, carbon monoxide, *Acinetobacter* isolate, carboxydobacteria

Carbon monoxide dehydrogenase (CD-DH) is an enzyme responsible for the oxidation of CO to CO_2 in several Gram-positive and negative carboxydobacteria which are able to grow aerobically with CO as a sole carbon and energy source (Hegeman, 1984; Kim and Hegeman, 1983*a*; Meyer and Fiebig, 1985; Meyer and Rohde, 1984, Meyer and Schlegel, 1983). Studies on the CO-DHs from several carboxydobacteria revelaed that those enzymes share several common biochemical, molecular, and immunological properties (Hegeman, 1984; Kim and Hegeman, 1983*a*; Kim and Kim, 1984; Meyer and Fiebig, 1985; Meyer and Rohde, 1984; Meyer and Schlegel, 1983).

Acinetobacter sp. 1 is a novel carboxydobacterium which does not require molybdenum for growth with CO (Cho *et al.*, 1985). Since several carboxydobacteria are known to depend on molybdenum for CO-autotrophic growth and have molybdenum as a functional cofactor in their CO-DHs (Meyer and Fiebig, 1985; Meyer and Rohde, 1984; Meyer and Schlegel. 1983), the ability of *Acinetobacter* sp. 1 to grow with CO in the absence of molybdenum implied that the bacterium may have another kind of CO-DH which is different from those of other carboxydobacteria.

Several basic properties of CO-DH in crude cell extracts of *Acinetobacter* sp. 1 were examined in

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this study to compare them with those of other well-studied CO-DHs and to provide more diverse information on the carboxydobacterial CO-DH.

MATERIALS AND METHODS

Organism and cultivation

Acinetobacter sp. 1 which was isolated from soils in Seoul, Korea (Cho *et al.*, 1985) was grown CO-autotrophically in mineral medium under the conditions described in a previous paper (Kim and Hegeman, 1981). Cells were harvested in mid-exponential growth phase and washed once in 0.05 M Tris-HCl buffer (pH 7.5, standard buffer) before cell extracts preparation.

Preparation of cell extracts

Cells were resuspended in cold standard buffer and disrupted by sonic treatment at 0°C as described previously (Kim and Hegeman, 1981). The solution was centrifuged at 10,000 × *g* for 30 min and the resulting supernatant fluids were used as crude cell extracts.

Enzyme assay

CO-DH activity was assayed photometrically at 30°C by measuring the CO-dependent reduction of thionin dye (Sigma) at 595 nm using anaerobic cuvettes and a Hitachi 200-20 spectrophotometer (Kim and Hegeman, 1981).

Electrophoresis

Non-denaturing polyacrylamide gel electrophoresis (PAGE) of the cell extracts was performed in gels containing 7.5% acrylamide by the method of Laemmli (1970), but without sodium dodecyl sulfate (SDS) as described previously (Kim and Hegeman, 1981). Enzymes were stained by activity staining using phenazine methosulfate (PMS) and nitro blue tetrazolium (NBT) under an atmosphere of CO or H_2 (Kim and Hegeman, 1981).

Immunodiffusion test

Double immunodiffusion assays were carried out in 1.2% agarose (Sigma, type V) gel by the method of Ouchterlony and Nilsson (1978) as described in a previous report (Kim *et al.*, 1982) except that immunoprecipitates were stained with 0.25% Coomassie brilliant blue R-250.

Protein determination

Proteins in crude cell extracts were determined by modified biuret reaction (Gornall *et al.*, 1949) after boiling the extracts in 20% NaOH for 10min (Meyer and Schlegel, 1978).

Chemicals, biochemicals, and gases

NAD, NADP, FAD, FMN, methylene blue, and 2,6-dichlorophenol-indophenol (DCPIP) were purchased from Sigma Chemical Co. CO (99.5%, vol/vol), H_2 (99.0%, vol/vol), and N_2 (99.99%, vol/vol) were obtained from Dongjin Gas Co., Korea. Other materials on high purity were obtained from usual commercial sources.

RESULTS

Inducibility and localization of CO-DH

Cells grown heterotrophically with 0.2% sodium succinate did not show any measurable CO-DH activity. The enzyme activity (22.4 μmol thionin reduced/mg protein/min) was detected only in cell extracts prepared from cells grown with CO. When the crude cell extracts were sedimented for 90 min at 100,000 × *g*, the CO-DH activity was found only in the soluble fractions; there was no CO-DH activity detectable in the particulate fractions washed once with the standard buffar (Table 1).

Artificial electron acceptors

The range of possible electron acceptors and the best reacting acceptor for the oxidation of CO were determined using the standard enzyme assay method except that thionin was substituted with testing acceptors. The crude cell extracts of *Acinetobacter* sp. 1 catalyzed the oxidation of CO with thionin, methylene blue, or DCPIP, but not with NAD, NADP, FAD, or FMN. The reduction rate was the highest when thionin or methylene

Table 1. *Localization of CO-DH in cells of Acinetobacter sp. 1 grown with CO*[a]

Fractions	Specific activity[b]
Crude cell extracts	22.4
Soluble fractions	25.5
Particulate fractions	< 0.003

[a]Enzyme activity in each fraction was measured using the standard enzyme assay method.
[b]μmol thionin reduced/ mg protein/min

blue was used as the acceptor. DCPIP was reduced at 27% of this rate. Therefore, the CO-DH was assayed by measuring the rate of reduction of thionin at 595 nm in the following studies as Kim and Hegeman (1981).

pH dependence and stability

The Co-DH was most active at pH 7.5 in 0.05M Tris-HCl buffer (Fig. 1). When 0.05M phosphate buffer was tested, the enzyme activity was maximal at pH 6.8. When stored at 4°C for 24h, the enzyme was most stable at pH 7.5 and 6.8 in 0.05 M of Tris-HCl and phosphate buffer, respectively (data not shown). These results are comparable to those of *Pseudomonas carboxydohydrogena* (Kim and Hegeman, 1983*b*) and *Pseudomonas carboxydovorans* (Meyer and Schlegel, 1979 and 1980). From the coincidence of pH value for maximal activity with that for stability, 0.05 M Tris-HCl (pH 7.5) was selected as the standard buffer for enzyme assay and for crude cell extracts preparation.

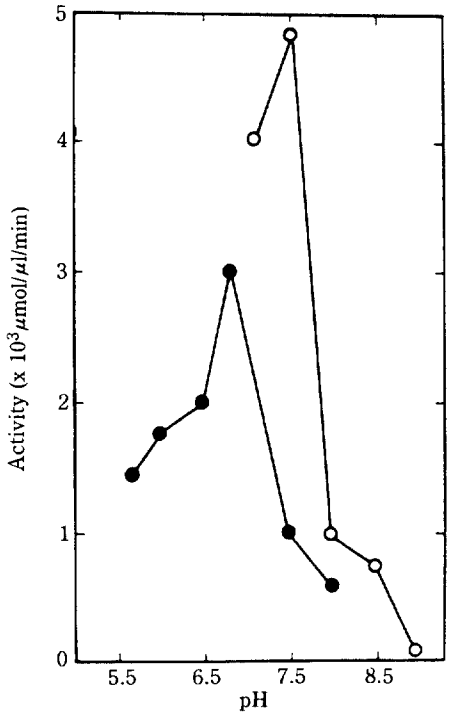

Fig. 1. *Effect of pH on the CO-DH activity. The optimal pH for the CO-DH activity was determined by the standard assay using various pH buffers of 0.05 M Tris-HCl (-○-) and 0.05 M phosphate (-●-).*

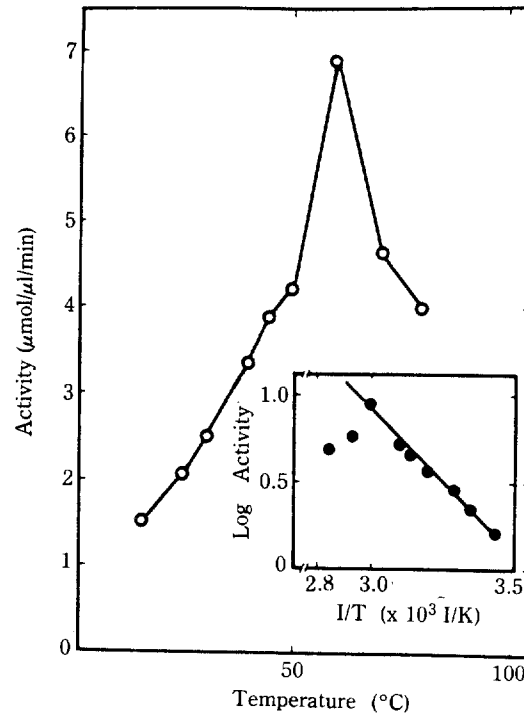

Fig. 2. *Effect of temperature on the CO-DH activity. Temperature dependence of the enzyme activity was tested at various temperatures using the standard assay method. Activation energy = 6.1 kcal/mol (25.5 kJ/mol).*

Temperature dependence and stability

Maximum CO-DH activity was found at 60°C (Fig. 2). This temperature, however, was not used for routine enzyme assay since almost 50% of the initial activity was inactivated in 15 min at this temperature. The enzyme was quite stable at 30°C and this temperature was used for the standard assay, although the oxidation of CO at 30°C proceeded three times slower than that at 60°C. The temperature dependence of the reaction rate followed the Arrhenius equation with an activation energy of 6.1 kcal/mol (25.5kJ/mol); the energy is lower than those of *P. carboxydohydrogena* (9.0 kcal/mol) (Kim and Hegeman, 1981) and *P. carboxydovorans* (8.6-8.8 kcal/mol) (Meyer and Schlegel, 1979 and 1980) but is higher than that of *Bacillus schlegelii* (1.24 kcal/mol) (Krüger and Meyer, 1984).

Enzyme kinetics

The standard assay method was used to measure the dependence of thionin reduction rate

on CO concentration to determine the K_m as described previously (Kim and Hegeman, 1981). The rate of thionin reduction followed the Michaelis-Menten kinetics. The K_m for CO turned out to be 154 μM following Lineweaver-Burk (1934) plot, which is higher than those of *P. carboxydohydrogena* (63 μM) (Kim and Hegeman, 1981) and *P. carboxydovorans* (45-53μM) (Myeyer and Schlegel, 1979 and 1980). The solubility of CO at 30°C under atmospheric pressure was taken the be 19.15μl in 1ml of water (Stephen and Stephen, 1963).

Effects of chelating agents, divalent cations, and metabolites

Several divalent cations were tested for their effects on the CO-DH activity. None of the metals tested (Se^{2+}, Mn^{2+}, Co^{2+}, Mg^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+}) at 1 mM concentration, except Mg^{2+} and Ba^{2+}, stimulated the enzyme activity; Mg^{2+} and Ba^{2+} increased the initial activity by 14% and 10%, but the failure of EDTA, KCN, and NaN_3 at 1 mM to inhibit the enzyme raises a question as to whether this enzyme has an easily removalbe metal cofactor. Cu^{2+} eliminated the enzyme activity completely, suggesting inactivation of a necessary sulfhydryl group for the enzyme activity by this ion. However, incubation of the enzyme in the presence of 1 mM iodoacetamide did not affect the enzyme activity (data not shown). Glucose and succinate at 6 mM inactivated 53% and 20% of the initial CO-DH activity, respectively, suggesting metabolite inhibition of the enzyme.

Hydrogenase and formate dehydrogenase activity

Hydrogenase and formate dehydrogenase activity in cell extracts of CO-grown *Acinetobacter* sp. 1 were determined adapting the standard assay method except that hydrogen gas and 0.5mM sodium formate were used as the substrate, respectively. Hydrogenase activity (33.6 μmol thionin reduced/mg protein/min) was found to be 1.5 times that of the CO-DH. Activity staining after non-denaturing PAGE of the cell extracts revealed that some of the hydrogenase activity is associated with the CO-DH (Fig. 3A). The cell extracts also contained formate dehydrogenase activity amounting to 20% of the CO-

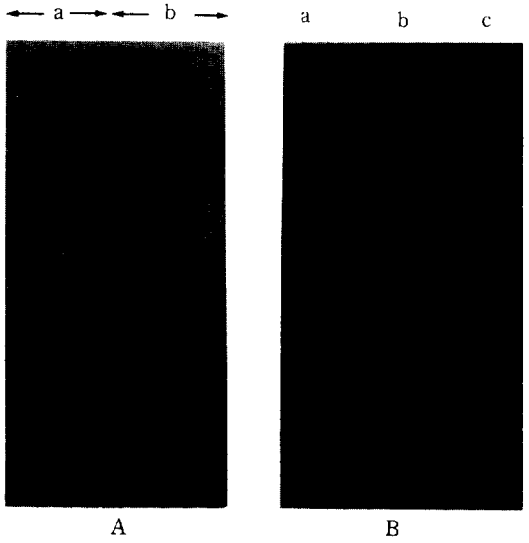

Fig. 3. **A,** *Hydrogenase activity in CO-DH. Activity staining after non-denaturing PAGE (7.5% acrylamide) with crude cell extracts of Acinetobacter sp. 1 was carried out with CO (a) or H_2 (b) as substrate.*
B, *Mobility of CO-DHs from several carboxydobacteria. Non-denaturing PAGE (7.5% acrylamide) was carried out with crude cell extracts of P. carboxydohydrogena (a), P. carboxydovorans (b), and Acinetobacter sp. 1 (c) followed by activity staining with CO as substrate. The bars represent 1 cm of migration.*

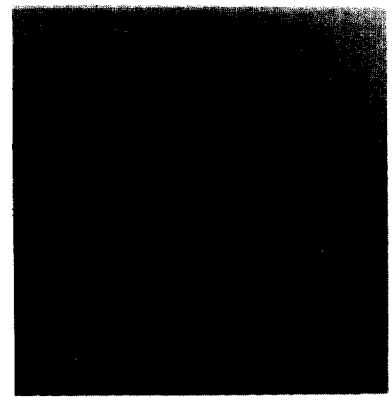

Fig. 4. *Double-immunodiffusion pattern for CO-DH from Acinetobacter sp. 1. Immunodiffusion assay was performed in 1.2% agarose gel for 24 h at 30°C followed by staining with Coomassie brilliant blue. Antiserum prepared against purified CO-DH of P. carboxydohydrogena, 6 μl (a); soluble fractions from CO-autotrophically grown cells of P. carboxydohydrogena, 15 μg (b), P. carboxydovorans, 17.5 μg (c), and Acinetobacter sp. 1, 20.5 μg (d).*

DH.

Molecular and immunological property

It was found that CO-DH in *Acinetobacter* sp. 1 migrates more rapidly in non-denaturing polyacylamide gel than those of *P. carboxydohydrogena* and *P. carboxydovorans* (Fig. 3B). Double immunodiffusion revealed that cell extracts prepared from cells of *Acinetobacter* sp. 1 grown with CO does not not cross-react with antiserum raised against purified CO-DH of *P. carboxydohydrogena* (Fig. 4).

Loss of CO-DH activity

Although the CO-DH of *Acinetobacter* sp. 1 was most stable at pH 7.5 in 0.05 M Tris-HCl when stored aerobically for 24h at 4°C, almost 90% of the enzyme was inactivated under this condition. The cell extracts retained only 30% of the initial CO-DH activity after 24h of incubation at -20°C. The remaining activities were stable for a week under each condition.

DISCUSSION

Among several known carboxydobacteria, *P. carboxydohydrogena, P. carboxydovorans, Pseudomonas carboxydoflava,* and *B. schlegelii* have been extensively studied with regard to the CO-DH in crude cell extracts or in purified systems (Hegeman, 1984; Kim and Hegeman, 1983*a* and *b*; Kim and Kim, 1984; Krüger and Meyer, 1984; Meyer and Fiebig, 1985; Meyer and Rohde, 1984; Meyer and Schlegel, 1983).

As in most carboxydobacteria, except in *P. carboxydoflava* (Kiessling and Meyer, 1982), the CO-DH in *Acinetobacter* sp. 1 was found to be a CO-inducible enzyme. Although the enzyme activity was detected exclusively in the soluble fractions after ultracentrifugation of the crude cell extracts, it could not be considered that the enzyme is a soluble one since we found in another experiment using spheroplasts of *Acinetobacter* sp. 1 that the enzyme may be loosely attached to the inner face of the cytoplasmic membrane (unpublished data) as that of *P. carboxydovorans* (Rohde *et al.*, 1984) and *Rhodopseudomonas gelationosa* (Wakim and Uffen, 1983). Detection of particulate hydrogenase activity in the CO-DH band after activity stainign with H_2 and PMS-NBT of polyacrylamide gel in the absence of NAD also supported this conclusion.

The crude CO-DH can use several dyes which have redox potentials between +10 mV and +217 mV as electron acceptors. The range of artificial electron acceptors is similar to that of the *P. carboxydovorans* enzyme (Meyer and Schlegel., 1979 and 1980) but is different from that of the *P. carboxydohydrogena* enzyme; *P. carboxydohydrogena* CO-DH cannot use DCPIP as an electron acceptor for the oxidation of CO (Kim and Hegeman, 1981 and 1983*b*). The present result suggests that a quinone or *b*-type cytochrome may serve as a physiological electron acceptor during CO oxidation. We found, however, in another test that the CO-DH of *Acinetobacter* sp. 1 could not catalyze the reduction of ubiquinone Q_{10} (Kim and Cho, 1986).

A relatively high temperature (60°C) for maximal CO-DH activity in *Acinetobacter* sp. 1, which is a mesophilic bacterium, suggests that the enzyme may have a metal cofactor. Although several chelators of divalent metals did not inactivate the CO-DH, stimulation of the enzyme by Mg^{2+} and Ba^{2+} supports this suggestion. However, the enzyme may not have the molybdenum cofactor which is present in the CO-DHs of *P. carboxydohydrogena, P. carboxydovorans, P. carboxydoflava,* and *B. schlegelii* (Meyer and Fiebig, 1985; Meyer and Rohde, 1984, Meyer and Schlegel, 1983) since *Acinetobacter* sp. 1 does not depend on molybdenum for CO-autotrophic growth (Cho *et al.*, 1985)

Meyer and Schlegel (1983) and Meyer and Rajagopalan (1984) reported that aerobic treatment of CO-DH from *P. carboxydovorans* with selenite activates the enzyme. However, selenite did not stimulate the enzyme in *Acinetobacter* sp. 1 in this experiment, suggesting that selenite plays no role in the oxidation of CO in *Acinetobacter* sp. 1.

Association of particulate hydrogenase activity with the CO-DH is a similar phenomenon to those of *P. carboxydohydrogena* (Kim and Hegeman,

1981) and *P. carboxydovorans* (Meyer and Schlegel, 1980) and hints an assumption that the CO-DH is a membrane-bound enzyme as discussed earlier. The occurrence of hydrogenase activity in CO-grown cell also suggests that *Acinetobacter* sp. 1 may be a hydrogen bacterium like most other carboxydobacteria(Kim and Hegman, 1983*a*, Meyer and Rohde, 1984; Meyer and Schlegel, 1983; Zavarzin and Nozhevnikova, 1977) even though we have not tried to grow this bacterium with H_2 and CO_2.

It has been reported that CO-DH of *Alcaligenes carboxydus* in non-denaturing polyacrylamide gel migrates more rapidly than those of other carboxydobacteria due to its low molecular weight (Cypionka *et al.*, 1980). Altough we are unable to calculate the presise molecular size of a protein in the usual non-denaturing polyacrylamide gel, the rapid migration of *Acinetobacter* sp. 1 enzyme suggests that it may have a lower molecular weight like that of *A. carboxydus* than those of *P. carboxydohydrogena* and *P. carboxydovorans*. According to the present data, CO-DH in *P. carboxydohydrgena* seems to be larger than that of *P. carboxydovorans*. This notion is supported by those of Kim *et al.*, (1982) and Kim and Kim (1984) that the size of the β subunit of the *P. carboxydohydrogena* enzyme is slightly larger than that of *P. carboxydovorans* but those of other kinds of subunits from the two strains are equal.

CO-DHs in most carboxydobacteria, expept in *B. schlegelii* (Krüger and Meyer, 1984). share a close immunological relationship (Kim *et al.*, 1982; Meyer and Fiebig, 1985; Meyer and Rohde, 1984; Meyer and Schlegel, 1983). However, CO-DHs in *Acinetobacter* sp. 1 and *P. carboxydohydrogena* were found to have no antigenic sites in common, implying that *Acinetobacter* sp. 1 enzyme may not share any common immunological properties with those of other carboxydobacteria.

Carboxydobacterial CO-DHs are known to be quite stable at low temperatures (Kim and Hegeman, 1981; Meyer and Schegel, 1979 and 1980). The enzyme in *Acinetobacter* sp. 1, however, was very sensitive to the incubation at low temperatures. Dithiothreitol, EDTA, cysteine, and phenylmethylsulfonylfluorids were ineffective in preventing the inactivation.

From all the results mentioned above, we are able to conclude that *Acinetobacter* sp. 1 has a novel CO-DH which has many properties different from those of other carboxydobacterial CO-DHs studied to date. Considering the unique CO-DH in thermophilic *Bacilli* (Krüger and Meyer, 1984), the present results suggest that there have been several independent line of CO-DH evolution in aerobic carboxydobacteria.

적 요

일산화탄소를 이용하여 자가영양적으로 성장한 ***Acinetobacter*** sp. 1 의 세포추출액은 혐기성 실험조건하에서 thionin, methylene blue, 2,6-dichlorophenol-indophenol 등을 일산화탄소의 산화를 위한 전자수용체로 사용할 수 있었으나 NAD, NADP, FAD, 또는 FMN등은 전자수용체로 이용하지 못하였다. 이 세균에 존재하는 일산화탄소 산화효소는 유도효소로 밝혀졌고, pH 7.5와 60℃에서 최대의 활성을 나타내었다. 이 효소의 활성화에너지는 6.1 kcal/mol (25.5 kJ/mol) 이며 일산화탄소에 대한 K_m값은 154μM로 밝혀졌다. 그리고 잘 알려진 몇가지 금속 chelating agent와 2가의 양이온들은 이 효소의 활성에 거의 영향을 미치지 않았는데, Cu^{2+} 이온만은 이 효소의 활성을 완전히 억제시켰다. 또한 이 효소는 포도당과 숙신산에 의해 활성이 저해되었으며, hydrogenase 의 활성도 나타내었다. 그리고 ***Acinetobacter*** sp. 1 의 일산화탄소 산화효소는 ***Pseudomonas carboxydohydrogena***의 일산화탄소 산화효소와 면역학적인 연관성이 없는 것으로 나타났다.

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REFERENCES

1. Cho, J.W., H.S. Yim, and Y.M. Kim, 1985. *Acinetobacter* isolates growing with carbon monoxide. *Kor. J. Microbiol.* **23,** 1-8.
2. Cypionka, H., O. Meyer, and H.G. Schlegel, 1980. Physiological characteristics of various speices of strains of carboxydobactera. *Arch. Microbiol.* **127,** 301-307.
3. Gornall, A.G., C.J. Bardawil, and M.M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177,** 751-766.
4. Hegeman, G.D., 1984. Oxidation of carbon monoxide by aerobic bacteria. *In* "Microbial growth on C_1 compounds" (Crawford, R.L., and R.S. Hanson, eds.), pp. 21-25. American Society for Microbiology, Washington, D.C.
5. Kiessling, M., and O. Meyer, 1982. Profitable oxidation of carbon monoxide or hydrogen during heterotrophic growth of *Pseudomonas carboxydoflava. FEMS Microbiol. Letts.* **13,** 333-338.
6. Kim, Y.M., and J.W. Cho. 1986. Electron transport system for carbon monoxide oxidation in *Acinetobacter* sp. 1. Kor. J. Microbiol. **24,** 46-50.
7. Kim, Y.M., and G.D. Hegeman, 1981. Purification and some properties of carbon monoxide dehydrogenase from *Pseudomonas carboxydohydrogena. J. Bacteriol.* **148,** 904-911.
8. Kim, Y.M., and G.D. Hegeman, 1983a. Oxidation of carbon monoxide by bacteria. *Intl. Rev. Cytol.* **81,** 1-32.
9. Kim, Y.M., and G.D. Hegeman, 1983b. Oxidation of carbon monoxide by *Pseudomonas carboxydohydrogena. Kor. J. Microbiol.* **21,** 27-35.
10. Kim, E.S., and Y.M. Kim, 1984. Subunit structure of carbon monoxide oxidase from *Pseudomonas carboxydovorans. Kor. Biochem. J.* **17,** 141-147.
11. Kim, Y.M., S. Kirkconnell, and G.C. Hegeman, 1982. Immunological relationships among carbon monoxide dehydrogenases of carboxydobacteria. *FEMS Microbiol. Letts.* **13,** 219-223.
12. Krüger, B., and O. Meyer, 1984. Thermophilic *Bacilli* growing with carbon monoxide. *Arch. Microbiol.* **139,** 402-408.
13. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriphage T_4. *Nature* **227,** 680-685.
14. Lineweaver, H., and D. Burk, 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56,** 658-666.
15. Meyer, O., and K. Fiebig, 1985. Enzymes oxidizing carbon monoxide. *In* "Gas enzymology" (Degn, H. *et al.*, eds.), pp. 147-168. Reidel D. Pub. Co.
16. Meyer, O., and K.V. Rajagopalan, 1984. Selenite binding to carbon monoxide oxidase from *Pseudomonas carboxydovorans.* Selenium binds covalently to the protein and activates specifically the CO-methylene blue reaction. *J. Biol. Chem.* **259,** 5612-5617.
17. Meyer, O., and M. Rohde, 1984. Enzymology and bioenergetics of carbon monoxide oxidizing bacteria. *In* "Microbial growth on C_1 compounds", (Crawford, R.L., and R.S. Hauson, eds.) pp. 26-33. American Society for Microbiology, Washington, D.C.
18. Meyer, O., and H.G. Schlegel, 1978. Reisolation of carbon monoxide utilizing hydrogen bacterium *Pseudomonas carboxydovorans* (Kistner) comb. nov. *Arch. Microbiol.* **118,** 35-43.
19. Meyer, O., and H.G. Schlegel, 1979, Oxidation of carbon monoxide in cell extracts of *Pseudomonas carboxydovorans. J. Bacteriol.* **137,** 811-817.
20. Meyer, O., and H.G. Schlegel, 1980. Carbon monoxide:methylene blue oxidoreductase from *Pseudomonas carboxydovorans. J. Bacteriol.* **141,** 74-80.
21. Meyer, O., and H.G. Schlegel, 1983. Biology of carbon monoxide-oxidizing bacteria. *Ann. Rev. Microbiol.* **37,** 277-310.
22. Ouchterlony, O., and L.A. Nilsson, 1978. Immunodiffusion and immunoelectrophoresis. *In* "Handbook of experimental immunology" (Weir, D.M., ed.), pp. 19.1-19.44. Blackwell

Scientific Publication, Oxford.
23. Rohde, M., F. Mayer, and O. Meyer, 1984. Immunocytochemical localization of carbon monoxide oxidase in *Pseudomonas carboxydovorans*. *J. Biol. Chem.* **259,** 14788-14792.
24. Stephen, H., and T. Stephen, 1963. Solubilities of inorganic and organic compounds, Vol. 1, part 1, pp. 364. Pergamon Press. Elmsford, New York.
25. Wakim, B.T. and R.L. Uffen, 1983. Membrane association of the carbon monoxide oxidation system in *Rhodopseudomonas gelatinosa*. *J. Bacteriol.* **153,** 571-573.
26. Zavarzin, G.A., and A.N. Nozhevnikova, 1977. Aerobic carboxydobacteria. Microbial Ecol. **3,** 305-326.

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