Constitutive Expression of Carbon Monoxide Dehydrogenase in *Acinetobacter* sp. Strain JC1 DSM 3803

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Carbon monoxide dehydrogenase (CO-DH) was found to be present in *Acinetobacter* sp. strain JC1 grown on CO and also on methylotrophic and heterotrophic substrates, except for pyruvate and nutrient broth. The amounts of CO-DH in cells grown on methylamine, glucose, galactose, and succinate were comparable to that of the CO-grown cells. CO-DH activity, however, was not detected by the dye-linked assay method in cell extracts prepared from cells grown on organic substrates, except on ethanol and succinate. The activity was detected when the CO-DH was stained by activity using CO as a substrate. CO-DHs in cells grown on different substrates were found to be identical in immunological properties.

KEY WORDS □ carbon monoxide, carboxydobacteria, CO dehydrogenase, *Acinetobacter* sp. JC1

Carboxydobacteria are a group of Gram-positive and -negative bacteria which are able to grow aerobically with carbon monoxide (CO) as the sole source of carbon and energy (9, 13). Most of the bacteria with a few exceptions are also known to grow on H_2 and CO_2 as well (4, 9, 13). The bacteria, except *Streptomyces thermoautotrophicus* which grows only on CO and H_2/CO_2 (4), are facultative chemoautotrophs and are capable of utilizing organic substrates (9, 13).

CO oxidation in carboxydobacteria is mediated by the well-characterized CO dehydrogenase (CO-DH) (9, 13). The enzyme is induced by CO and is absent in cells grown heterotrophically, except for *Pseudomonas carboxydoflava* (2, 9, 12, 14).

In earlier works, the CO-DH of *Acinetobacter* sp. strain JC1, a newly isolated carboxydobacterium, was found to be CO-inducible like those of most other carboxydobacteria (2, 7). We, however, recently recognized through careful examination that the CO-DH of this bacterium is present in cells grown not only on CO but also on organic substrates.

In this study, we report the constitutive nature of CO-DH in *Acinetobacter* sp. strain JC1.

MATERIALS AND METHODS

Organism and cultivation

Acinetobacter sp. strain JC1 DSM 3803 (1) was grown autotrophically in standard mineral medium (8) with a gas mixture of 30% CO-70% air or 60% H_2-10% CO_2-30% O_2. For heterotrophic growth, the mineral medium was supplemented with appropriate concentrations of organic substrates. Growth was measured by turbidity determined at 436 nm using a spectrophotometer.

Preparation of cell-free extracts

Crude cell-free extracts were prepared in 0.05 M Tris-HCl (pH 7.5) as described previously (8). Protein contents in the extracts were determined by the biuret method after treatment with NaOH (5, 8).

CO-DH and CO-DH inhibitor assays

CO-DH activity in crude cell extracts was measured according to Kraut *et al.* (10). Inhibition of CO-DH by CO-DH inhibitor was assayed after Do *et al.* (3).

Electrophoresis and activity staining

Non-denaturing polyacrylamide gel electrophoresis (PAGE) was carried out by a modified method (8) of Laemmli (11) in the absence of sodium dodecyl sulfate (SDS). CO-DH was stained using an activity stain as described previously (8).

Immunodiffusion test

Double immunodiffusion assays were performed in 1.2% agarose gel for 24 h at 30°C by a modified method (3) of Ouchterlony and Nilsson (15).

RESULTS

Detection of CO-DH activity

The CO-DH activity was found only in cells grown on CO, H_2, ethanol, and succinate (Table 1). The activity was not detected from cells growing in any growth phases on methanol,

Table 1. *CO-DH in Acinetobacter sp. strain JC1*

Substrates	Concn (%)	CO-DH		
		Sp act[a]	Staining[b]	Amt (%)[c]
CO	30[f]	20.3	+	7.8
H_2	60[f]	6.3	+	3.0
Methane	30[f]	NT[e]	NT	NT
Methanol	0.5[d]	0.0	+	1.3
Formaldehyde	0.5[d]	NT	NT	NT
Formate	0.5[f]	NT	NT	NT
Methylamine	0.5[f]	0.0	+	5.6
Dimethylamine	0.5[d]	NT	NT	NT
Trimethylamine	0.5[d]	NT	NT	NT
Ethanol	0.5[d]	15.6	+	2.0
Isopropanol	0.5[d]	0.0	−	3.0
Acetate	0.5[f]	0.0	+	1.4
Pyruvate	0.5[f]	0.0	−	NT
Succinate	0.5[f]	3.0	+	6.8
Glucose	0.2[f]	0.0	+	5.7
Galactose	0.2[f]	0.0	+	5.7
Nutrient broth	0.8[f]	0.0	−	NT

[a] Micromoles of 2-(4-indophenyl)-3-(4-nitrophenyl)-2H-tetrazolium chloride reduced per milligram of protein per minute as determined according to Kraut *et al.* (10); [b] CO-DH was detected by an activity staining using CO as substrate (8); [c] % of the total soluble protein determined after densitometric analysis of the polyacrylamide gel (8); [d] V/V; [e] Not tested; [f] W/V.

methylamine, isopropanol, acetate, pyruvate, glucose, galactose, and nutrient broth.

Presence of CO-DH in cells grown on various substrates

The CO-DH of *Acinetobacter* sp. JC1 was found to be present in most cells except those grown on pyruvate and nutrient broth when activity staining was applied after nondenaturing PAGE (Table 1, Fig. 1 and 2). The amounts of CO-DH in cells growing at the early stationary phase on methylamine, glucose, galactose, and succinate were found to be comparable to that of the CO-grown cells after densitometric analysis of total soluble proteins electrophoresed on polyacrylamide gel (Table 1).

Immunological properties of CO-DHs in cells grown on various substrates

Double immunodiffusion revealed that CO-DHs in cell extracts prepared from cells grown on various substrates cross-react with antiserum raised against the purified CO-DH of *Acinetobacter* sp. JC1 grown on CO (Fig. 3). The lack of spurs between wells containing different cell-free extracts indicates that the antigenic site(s) of the CO-DHs are completely identical with that of the CO-grown cells.

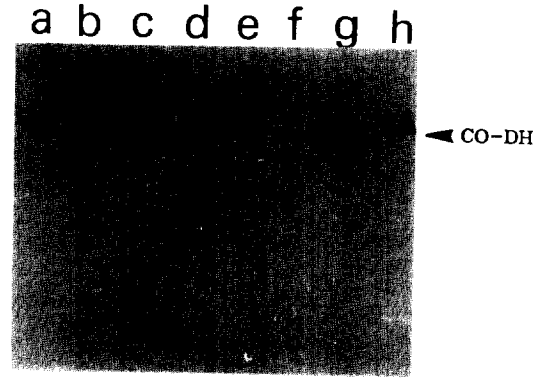

Fig. 1. *Activity staining for CO-DH in cells grown on different substrates.*
Activity staining after nondenaturing PAGE on 7.5% polyacrylamide gel was carried out using CO as a substrate. Each lane contains 80 μg of the crude extracts prepared from cells growing at the early stationary phase on glucose (lane a), acetate (lane b), galactose (lane c), isopropanol (lane d), CO (lane e), pyruvate (lane f), nutrient broth (lane g), and methylamine (lane h).

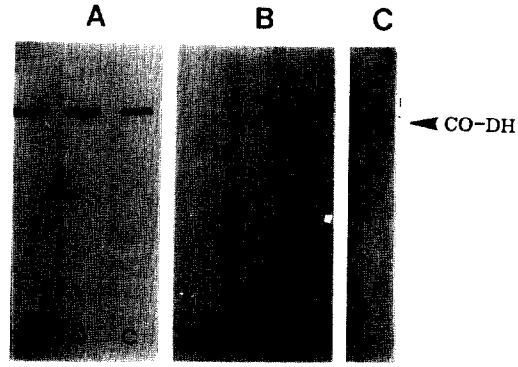

Fig. 2. *Activity staining for CO-DH in cells growing at different growth phases.*
Activity staining after nondenaturing PAGE (7.5% acrylamide) with 80 μg each of the crude extracts was carried out with CO as a substrate. Cell-free extracts were prepared from cells growing at the mid-log (lane a), early stationary (lane b), and late stationary (lane c) phases on methanol (A) and pyruvate (B). C is the extracts from CO-grown cells.

DISCUSSION

The presence of CO-DH in cells of *Acinetobacter* sp. JC1 grown on organic substrates, except on

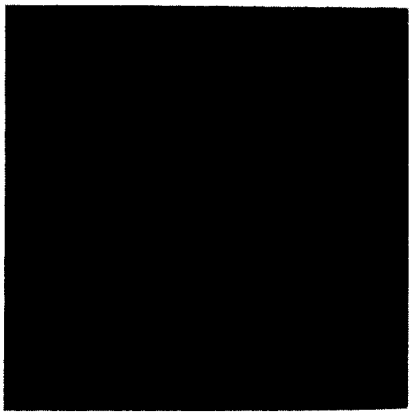

Fig. 3. *Double immunodiffusion patterns for CO-DH in cells grown on different substrates.*
AS is the antiserum raised against purified CO-DH from *Acinetobacter* sp. strain JC1 (5 μl). Wells contain crude extracts prepared from cells grown on nutrient broth (20 μg; 1), methanol (25 μg; 2), CO (10 μg; 3), glucose (15 μg; 4), galactose (7 μg; 5), and succinate (5 μg; 6).

pyruvate and nutrient broth, indicates that the enzyme in this bacterium is not inducible and is expressed constitutively as in *P. carboxydoflava* (9, 14). The CO-DH of *Acinetobacter* sp. JC1 has been considered as a CO-inducible enzyme (2, 7), which is contradictory to the present results. The earlier studies, however, should not be considered as an error since the results were obtained from experiments done by enzyme assay using cell-free extracts prepared from cells grown on nutrient broth (2) or glucose (7). Absence of CO-DH in cells grown on pyruvate or nutrient broth suggests that the substrates or certain metabolite(s) of the substrates may repress expression of the CO-DH genes. Detection of CO-DH activity by the staining technique, but not by the enzyme assay, in cells grown on several sustrates including methanol and glucose implies that inhibitor of CO-DH may be involved in this interesting phenomenon as suggested by Do *et al.* (3) in *Pseudomonas carboxydovorans*, i.e. CO-DH activity was inhibited by the CO-DH inhibitor during enzyme assay, but the enzyme was not influenced by the inhibitor when it was subjected to stain by activity. It was found in this study that the inhibitory activity against CO-DH is present in cell-free extracts of *Acinetobacter* sp. JC1 prepared from cells grown on methanol, glucose, methylamine, pyruvate, isopropanol, galactose, acetate, and nutrient broth (data not shown).

It has been reported that CO-DH activity of *P. carboxydoflava* was detected by enzyme assay in cells grown with pyruvate (0.4%, v/v) and glucose (0.8%, v/v) (6). This together with the present results and the report that the CO-DH of *P. thermocarboxydovorans* which is inducible by CO was detected by enzyme assay from cells grown on pyruvate under carbon-limited condition (less than 0.3%, w/v) (12) implies that the mechanism for expression of CO-DH and regulation of the enzyme activity is complex and may differ among types of carboxydobacteria. The constitutive expression of CO-DH in *Acinetobacter* sp. JC1 during growth not only on CO but also on H_2 and a variety of organic substrates as an energy source supports the possibility suggested before that this organism may play an important role in the removal of CO in natural environments (7).

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초 록: *Acinetobacter* sp. Strain JC1 DSM 3803에 존재하는 일산화탄소 산화효소의 구성적 발현

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일산화탄소 산화효소는 일산화탄소 뿐만 아니라 다른 일탄소화합물 및 pyruvate와 nutrient broth를 제외한 타가영양적 성장기질에서 *Acinetobacter* sp. strain JC1이 성장할 때에도 항상 존재하였다. Methylamine, glucose, galactose 및 succinate 등에서 성장한 세균에 존재하는 일산화탄소 산화효소의 양은 일산화탄소에서 성장한 세균에 존재하는 효소의 양과 비슷한 수준이었다. 그러나 ethanol과 succinate를 제외한 다른 유기물에서 성장한 세균의 일산화탄소 산화효소의 활성은 염색약을 이용한 효소활성측정법으로는 측정할 수 없었다. 이러한 활성은 단지 일산화탄소를 기질로 사용하여 일산화탄소 산화효소를 활성염색했을 때만 나타났다. 서로 다른 기질을 이용하여 성장한 세균들에 존재하는 일산화탄소 산화효소들은 면역학적 성질이 동일한 것으로 밝혀졌다.