KOR. JOUR. MICROBIOL. February. 1986 p. 46-50 Vol. 24, No. 1

Electron Transport System for Carbon Monoxide Oxidation in *Acinetobacter* sp. 1

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Acinetobacter sp. 1의 일산화탄소 산화를 위한 전자전달계

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Experiments with particulate fractions of *Acinetobacter* sp. 1 revealed that coeuzyme Q_{10} is not the physiological electron acceptor, and that cytochromes of *a*, *b*, *c*, and *o* types are found in cells grown with carbon monoxide (CO) as the sole source of carbon and energy. It was found that cytochromes of *b* and *o* types, but not the *a* and *c* types, are functional in CO oxidation. Nicotinamide adenine dinucleotide (phosphate) is not involved in CO oxidation.

Carboxydobacteria are strictly aerobic which can use carbon monoxide (CO) as a sole source of carbon and energy and contain cytochromes of *b*, *c*, *a*, and *o* types (Cypionka and Meyer, 1983a and b; Kim and Hegeman, 1981b and 1983a; Kirkconnell, 1978; Meyer, 1985; Meyer and Schlegel, 1978 and 1983; Meyer and Rohde, 1984; Zavarzin and Nozhevnikova, 1977).

Studies of artificial electron acceptors for CO oxidation with purified and crude CO dehydrogenases (CO-DHs) from several carboxydobacteria (Cho, 1983; Cypionka *et al.*, 1980; Kim and Hegeman, 1981a and 1983b; Kirkconnell, 1978; Meyer and Schlegel, 1979 and 1980), restoration of the CO-oxidizing activity in ultraviolet-treated extracts of *Pseudomonas carboxydohydrogena* with coenzyme Q_{10} (UQ_{10}) (Kim and Hegeman, 1981b), and detection of UQ_{10} from CO-grown *Pseudomonas carboxydovorans* (Meyer and Schlegel, 1980) suggested that ubiquinone is a possible physiological electron acceptor for CO oxidation in carboxydobacteria.

The present work was carried out to learn whether the typical cytochrome system found in other carboxydobacteria is present and functions in *Acinetobacter* sp. 1 (Cho *et al.*, 1985) during growth on CO and to determine whether electrons from CO are delivered at the level of quinone. The questions were approached by analysis of visible light spectra of particulate fractions, by measuring the effects of several electron transport system (ETS) inhibitors, and by testing the ability of CO-DH to use UQ_{10} as an electron acceptor.

MATERIALS AND METHODS

Organism and cultivation

Acinetobacter sp. 1 was isolated from soil in Seoul, Korea by Cho *et al.* (1985). The cell was grown in liquid mineral medium with 30% CO

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(99.5% minimum, vol/vol, Ulsan Gas Co.) as the carbon and energy source as described before (Kim and Hegeman, 1981a). Growth was determined by measuring turbidity at 436 nm using a Hitachi 200-20 spectrophotometer. Cells were harvested at the late exponential growth phase by centrifugation for 20 min at 10,000 x *g*, washed once with 0.05M Tris-hydrochloride (pH 7.5, standard buffer), and stored at #-20°C.

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, 1981a). The solution was centrifuged at 10,000 x *g* for 30 min at 4°C and the resulting supernatant fluids were used as crude cell extract. The crude extract was then centrifuged at 100,000 x *g* for 90 min at 4°C. The sediment was washed by resuspension in cold standard buffer and recentrifuged. The resulting sediment was used as particulate fraction.

Assay for ubiquinone reduction

The ability of CO-DH in crude cell extracts of *Acinetobacter* sp. 1 to use ubiquinone as physiological electron acceptor was tested by the assay for CO-DH as described by Kim and Hegeman (1981a) except that thionin was substituted with UQ_{10} (bovine heart, Sigma, St. Louis, U.S.A.). UQ_{10} was dissolved in absolute ethanol.

Spectral analysis of cytochromes

Particulate fraction obtained from crude extract was suspended in 30% bovine serum albumin (Sigma, St. Louis, U.S.A.) and a drop of 30% H_2O_2 was added to oxidize, or a few crystals of sodium hydrosulfite to reduce the samples, respectively. To examine effects of cyanide and CO, a few crystals of NaCN were added, or the untreated extract and the extract reduced with sodium hydrosulfite were flushed with CO for 6 min, respectively. The absorption spectra of samples were recorded from 500 nm to 650 nm at room temperature using a Hitachi 200-20 spectrophotometer.

Oxygen consumption tests

Effects of several ETS inhibitors on the CO-dependent oxygen uptake of cell extracts were tested at 30°C using a YSI 53 biological oxygen monitor (Yellow Spring Instrument, Yellow Spring, U.S.A.) to determine which cytochromes are working in cells growing with CO. The inhibitors rotenone, thenoyltrifluoroacetone (TTFA), antimycin A, and 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) were from Sigma, St. Louis, U.S.A. and dissolved in absolute ethanol. NaCN was dissolved in the standard buffer. Each inhibitor was added singly to a portion of crude extract. The final concentration of the inhibitors was 4μM, except for NaCN (10 mM).

RESULTS AND DISCUSSION

UQ_{10} was not reduced

It was found that CO-DH of *Acinetobacter* sp. 1 does not reduce UQ_{10} with CO as substrate (data not shown), suggesting that the UQ_{10} may not act as physiological electron acceptor in this bacterium growing on CO. If we think that the enzyme of *P. carboxydohydrogena* which could not use 2, 6-dichlorophenol-indophenol (DCPIP) as an electron acceptor reduced UQ_{10} (Kim and Hegeman, 1981a and b) and that of *P. carboxydovorans* which reduced DCPIP, but not the UQ_{10} (Meyer, 1982; Meyer and Schlegel, 1980; Schlegel and Meyer, 1981), it may be possible to suggest that the physiological electron acceptor for CO oxidation in *Acinetobacter* sp. 1 is similar to that of *P. carboxydovorans* which has redox potential more positive than the UQ_{10}; the acceptor may be other kind of ubiquinone.

The nature of cytochromes

The cytochrome composition of *Acinetobacter* sp. 1 grown on CO was investigated by means of visible light difference spectra (Fig. 1). The reduced minus oxidized difference spectrum of the particulate fraction clearly disclosed the presence of cytochromes of *b* (peaks at 532,558, and 562 nm), *c* (peak at 550 nm), and *a* (peaks a 595 and 603 nm). The presence of *a* type cytochromes in this bacterium is contradict to the previous report (Cho *et al.*, 1985) that the bacterium revealed no oxidase activity. We, therefore, assume that the *a* type cytochromes detected in the spectral assay have

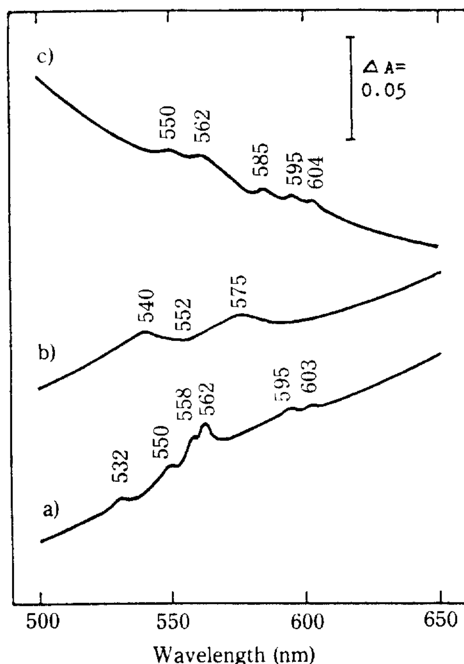

Fig. 1. *Absorption spectra of particulate fractions from Acinetobacter sp. 1. The absorption spectra of samples were recorded from 500 to 650 nm at room temperature using a Hitachi 200-20 spectrophotometer as described in methods.*
Symbols: reduced minus oxidized (a), CO minus reduced (b), and cyanide minus oxidized (c).

Table 1. *Effects of inhibitors on oxygen consumption in cell extracts*[a]

Inhibitors	Conc. (μM)	Remaining activity (%)[b]
None (+CO)[c]		100
None (−CO)[c]		<0.002
Rotenone	4	100
Antimycin A	4	100
TTFA	4	63
HOQNO	4	60
NaCN	1×10^4	55

[a] O_2 consumption by each sample was measured at 30℃ using an oxygen monitor as described in methods.
[b] O_2 consumption without inhibitor (about 280μl/mg protein/h) after 1h at room temperature was set as 100%.
[c] O_2 consumption was measured using cell extracts which were not treated with inhibitors in the presence (+CO) and absence (−CO) of CO as substrate.

lost their ability to act as terminal oxidases for unknown reason(s).

After NaCN treatment, peaks at 532 and 558 nm disappeared and a new peak which represents cyanide-bound cytochrome oxidase appeared at 585 nm. The size of the peak at 562 nm was reduced to some extent. When the particulate fraction was flushed with CO, peaks for cytochromes *b, c,* and *a* disappeared, and two new peaks (540 and 575 nm) and one trough (552 nm) appeared, strongly supporting the conclusion that cytochrome *o* which has a lower affinity for CO than cytochrome a_3 (Broberg and Smith, 1967; Van Verseveld *et al.*, 1983; Zannoni *et al.*, 1974) functions as a terminal oxidase in cells growing with CO.

The present result, together with those reports that cytochrome *o* is not only present but also functions in *P. carboxydohydrogena* (Cypionka and Meyer, 1983a; Kim and Hegeman, 1981b), *P. carboxydovorans* (Cypionka and Meyer, 1983a and b), and several other carboxydobacteria (Cypionka and Meyer, 1983a), implying that the cytochrome *o* may be an inevitable terminal oxidase for the respiratory metabolism of carboxydobacteria with CO as the sole energy source.

Effects of electron transport inhibitors on CO-dependent oxygen uptake

Several well-known ETS inhibitors were used to test whether the ETS found to be present in *Acinetobacter* sp. 1 functions in cells growing under CO. Among the inhibitors tested, TTFA, HOQNO, and cyanide were effective inhibitors of the CO-dependent oxygen uptake, but rotenone and antimycin A were found to be completely ineffective (Table 1).

The observation suggests that *c* type cytochrome is not involved in CO oxidation, which is the same in *P. carboxydovorans* (Cypionka and Meyer, 1983a; Meyer, 1985; Meyer and Schlegel, 1983), but not in *P. carboxydohydrogena* (Kim and Hegeman, 1981b and 1983a). The result also indicates that electrons are delivered from CO-DH to the ETS at the level of quinone and that NAD(P) is not involved during growth with CO, as is also true of those in other carboxydobacteria

(Cypionka and Meyer, 1983b; Kim and Hegeman, 1981b; Meyer, 1985; Meyer and Schlegel, 1983). It has been reported that cytochrome *o* seems to be less sensitive to cyanide inhibition than cytochrome a_3 (Lemberg and Barrett, 1973, Porte and Vignais, 1980), perhaps explaining why 10 mM NaCN was not sufficient to completely inhibit oxygen uptake.

All carboxydobacteria studied to date cannot obtain reducing power directly through the reduction of NAD(P) to NAD(P)H with CO as substrate. *Acinetobater* sp. 1 like other carboxydobacteria, must generate reduced pyridine nucleotide by reverse electron transport, a process which is inefficient compared with direct reduction of NAD(P) by substrate. This may explain the slow growth of *Acinetobacter* sp. 1 with CO (t_d = 19 h) as the sole source of carbon and energy (Cho *et al.*, 1985).

The work also revealed that the ETS which functions in *Acinetobacter* sp. 1 growing on CO resembles that of *P. carboxydovorans* in many aspects; they may share identical or very similar physiological electron acceptor, and cytochrome *c* is not involved in the oxidation of CO. However, it is not clear in this work whether the respiratory chain of *Acinetobacter* sp. 1 is branched as those of *P. carboxydovorans* (Cypionka and Meyer, 1983b; Meyer, 1985; Meyer and Schlegel, 1983) and *P. carboxydohydrogena* (Kim and Hegeman, 1981b and 1983a).

적 요

일산화탄소를 유일한 탄소 및 에너지원으로 이용하여 성장한 *Acinetobacter* sp. 1의 일산화탄소 산화를 위한 전자전달계를 조사하였다. 이 세균은 *a*, *b*, *c*, 그리고 *o*형의 사이토크롬을 소유하고 있었고, UQ 10은 이 세균의 생리적인 전자수용체로 작용할 수 없음이 밝혀졌다. 이 세균에 존재하는 사이토크롬들 중에서 *b*와 *o*는 일산화탄소 산화와 연관되어 있으나 *a*와 *c*는 관계가 없었으며, NAD(P)도 이 세균의 일산화탄소 산화와 무관한 것으로 밝혀졌다.

ACKNOWLEDGEMENT

This work was supported by a grant from Korea Science and Engineering Foundation (1982-1984).

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(Received Feb. 6, 1986)