ATP-GENERATION COUPLED WITH C_1-COMPOUND OXIDATION BY METHYLOTROPHIC BACTERIUM *PSEUDOMONAS* sp.2

A. I. NETRUSOV, Y. V. RODIONOV and E. N. KONDRATIEVA
Department of Microbiology, Moscow State University, Moscow, USSR

Received 20 December 1976

1. Introduction

In recent years different methylotrophs have been intensively investigated. Results have been summarized in some reviews [1–3], but up to now there has been no experimental data about ATP-formation coupled with C_1-substrate oxidation by microorganisms. The present communication describes a study of this problem in facultative methylotrophic bacterium *Pseudomonas* sp.2 [4] assimilation, with *iCl*⁻-serine pathway formaldehyde similar to *Pseudomonas* sp. M27 [5].

2. Materials and methods

Cultures of *Pseudomonas* sp.2 were grown in medium [6] containing methanol (0.3% v/v) on rotatory shaker (220 rev./min) at 30°C. Cells from cultures in exponential growth-phase were washed with cold Tris–HCl buffer (50 mM, pH 7.8) and suspended in solution, containing sucrose (200 mM), MgCl (5.0 mM) and dithiothreitol (1.0 mM). The cells suspension was disrupted with a X-press (LKB) and centrifugated at 20 000 × *g* for 15 min at 4°C. The membrane-fraction pelleted after crude-extract centrifugation at 165 000 × *g* for 1 h at 4°C. The membrane-containing pellet was washed in same vol. 50 mM Tris–HCl buffer (pH 7.8) with 5.0 mM $MgCl_2$, suspended in the same buffer.

Abbreviations: FP flavoprotein, FCCP fluorcarbonyl cyanide phenilhydrazone, Q_9 ubiquinone-9, cyt. *b*, *c*, $a + a_3$ cytochromes, PMS phenazine methosulfate

ATP-Formation was measured by fluorometer [7] according to $NADP^+$-reduction in a system containing, in 1.5 ml glass-distilled water: Tris–HCl buffer (pH 7.8) 200.0 μmol, KH_2PO_4 5.0 μmol, glucose 5.0 μmol, ADP 0.5 μmol, hexokinase (Serva, USA) 10.0 IU, glucose-6-phosphate dehydrogenase (Serva) 5.0 IU, $NADP^+$ 1.0 μmol, membrane-fraction 0.5–1.0 mg protein. Different organic substrates were added to membranes in concentration 10^{-3} M. NADH-generation system contained: lactate 5.0 mM, lactate dehydrogenase (Serva) 10.0 IU and NAD^+ 10^{-5} M. Ascorbate oxidation was mediated by horse-heart cytochrome *c* (Calbiochem, USA). The reduced cytochrome *c* was prepared by treatment with dithionite and subsequent dialysis. When formate and methanol were used as substrates for membrane-oxidation the systems were supplemented by formate dehydrogenase (10.0 IU) and NAD^+ (10^{-5} M) or methanol dehydrogenase (10.0 IU).

Formate- and methanol-dehydrogenases were got from cell-free extracts of methylotrophic bacterium by ammonium sulphate treatment followed by chromatography on the column of DE-52-cellulose. Samples of enzymes were homogenous according analytical centrifugation data. The respiratory activity of membranes in the presence of different substrates was measured polarographically in the same system which were used for estimation of ATP. Protein was measured by the method of Lowry et al. [8].

3. Results

The membrane fraction of *Pseudomonas* sp.2 cells, grown on methanol medium, reveals the capacity for

North-Holland Publishing Company – Amsterdam

Table 1
ATP-Generation coupled with different compound-oxidation by membrane-fraction of *Pseudomonas* sp. 2

Reaction system contains[a]	ATP ($nmol \cdot min^{-1} \cdot mg^{-1}$ protein)	O_2-uptake ($nmol \cdot min^{-1} \cdot mg^{-1}$ protein)	P : O
NADH-Generation system	4.50	2.60	1.71
NADH + FCCP	0.00	3.40	
NADH + antimycin A	0.10	0.30	
Formate	27.50	13.00	2.10
Formate + FCCP	0.75	15.00	
Formate + antimycin A	0.30	0.40	
Succinate	15.80	10.30	1.53
Succinate + FCCP	0.16	11.20	
Succinate + antimycin A	0.16	0.15	
Ascorbate + cyt. *c*	0.81	2.40	0.34
Ascorbate + FCCP	0.00	2.50	
Ascorbate + antimycin A	0.81	2.40	0.34
Reduced cyt. *c*	2.15	4.00	0.54
Reduced cyt. *c* + FCCP	0.00	4.20	
Reduced cyt. *c* + antimycin A	2.15	4.00	0.54
Methanol	7.40	14.20	0.52
Methanol + FCCP	0.02	16.10	
Methanol + antimycin A	7.40	14.20	0.52

[a] Reaction-system composition is described in Materials and methods

FCCP was used in concentration $2.1 \cdot 10^{-6}$ M, antimycin A, 5 μg/mg protein

respiration and ATP-synthesis in the presence of formate, methanol, as well as succinate, ascorbate, NADH or reduced cytochrome *c* (table 1). In all cases ATP-formation is inhibited by FCCP. This confirms its synthesis to be coupled with the function of electron-transport chain. At the same time O_2-uptake rate, in the presence of uncouplers such as FCCP, is somewhat increased, indicating respiratory control.

According to P:O ratios (1.54/2.0) the oxidation of one molecule of NADH, formate or succinate, is connected with generation of more energy (ATP) than oxidation of one molecule of methanol, reduced cytochrome *c* or ascorbate (P:O = 0.34/0.54).

ATP-Formation by membranes in the presence of methanol or reduced cytochrome *c* is not sensitive to antimycin A, but in the presence of formate, NADH or succinate, the process is inhibited (table 1).

It has been shown as well that partially purified cytochrome *c* of *Pseudomonas* sp.2 may be reduced in the presence of methanol and methanol dehydrogenase, but animal cytochrome *c* does not show any reduction.

4. Discussion

It was shown earlier, that *Pseudomonas* sp.2, similar to other methylotrophic bacteria, oxidized methanol to CO_2 as a result of action of PMS-dependent methanol dehydrogenase and then NAD-dependent formaldehyde- and formate-dehydrogenases [9,10]. The oxidation of formaldehyde may be also catalyzed by methanol dehydrogenase. According to enzyme activity this pathway seems to have a more important significance.

It was shown as well that respiratory activity of intact cells of *Pseudomonas* sp.2 in the presence of formate was inhibited by rotenone, atebrine and amytal. However, in the presence of methanol the inhibitory effect of these compounds was not observed [11]. This evidence suggests the participation of electron-transport chain in oxidation of formate by *Pseudomonas* sp.2 beginning from NADH. The same connection seems to exist in case of methylamine utilization by this microorganism because NAD-dependent *N*-methylglutamate dehydrogenase catalyzed its oxidation [10].

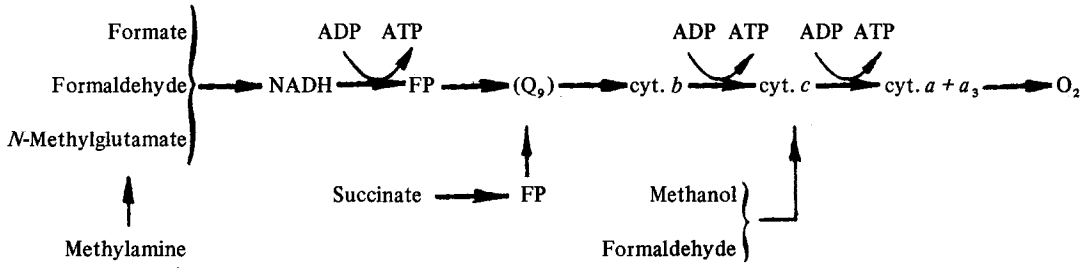

Fig.1. Possible scheme of electron-transport system of *Pseudomonas* sp.2 coupled with ATP-generation

The study of properties of methanol dehydrogenase of *Pseudomonas* sp. AM1 suggested its cytochrome *c* to be an electron-acceptor from methanol [12]. This suggestion is confirmed by the results of ATP-formation by membrane-fraction of *Pseudomonas* sp.2 as well as by action of inhibitors on this process and the respiratory activity of intact cells in the presence of methanol.

Thus, the earlier observations [10–13] and the presented data allow to propose following scheme of action of electron-transport chain coupled with ATP-generation in *Pseudomonas* sp.2 in case of oxidation of one-carbon and some other substrates (fig.1).

References

[1] Quayle, J. R. (1972) Adv. Microbiol. Physiol. 7, 119–203.
[2] Ribbons, D. W., Harrison, J. E. and Wadzinski, A. M. (1970) Ann. Rev. Microbiol. 24, 135–158.
[3] Anthony, C. (1975) Sci. Progr. 62, No. 246, 167–206.
[4] Kirikova, N. N. (1970) Microbiologia (USSR) 39, 7–14.
[5] Anthony, C. and Zatman, L. J. (1964) Biochem. J. 92, 609–614.
[6] Kaneda, T. and Roxburgh, J. (1959) Can. J. Microbiol. 5, 87–98.
[7] Borisov, A. Y., Iwanowskii, R. N. and Kondratieva, E. N. (1973) Biochimija 38, 59–62.
[8] Lowry, O., Rosebrough, J., Farr, A. and Randall, R. (1951) Biochem. J. 193, 265–275.
[9] Netrusov, A. I. (1973) Nauchnie dockladi vischei schkoli, biologitcheskie nauki No. 1, 99–102.
[10] Netrusov, A. I. (1975) Microbiologia 44, 552–554.
[11] Netrusov, A. I., Verchoturov, V. N., Kirikova, N. N. and Kondratieva, E. N. (1971) Microbiologia 40, 200–206.
[12] Anthony, C. (1975) Biochem. J. 146, 289–298.
[13] Kirikova, N. N., Trotzenko, Y. A. and Kondratieva, E. N. (1972) Microbiologia 41, 5–10.