Effect of Cyclopropane Treatment of *Methylosinus trichosporium* (OB3b) for Methane Hydroxylation

Masahiro Shimoda and Ichiro Okura*

Department of Bioengineering, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan

Methanol accumulation was observed by methane hydroxylation when *Methylosinus trichosporium* (OB3b) was treated with cyclopropane.

Methane mono-oxygenase catalyses *in vivo* hydroxylation of methane to produce methanol according to equation (1).[1]

$$CH_4 + NADH + H^+ + O_2 \rightarrow CH_3OH + NAD + H_2O \quad (1)$$

Methane mono-oxygenase from *Methylococcus capsulatus* (Bath)[2—5] and from *Methylosinus trichosporium* (OB3b)[6,7] have been purified and their enzymatic properties have been studied. However, whereas the purified mono-oxygenases are unstable and unsuitable for long-term use for methane hydroxylation, the bacterium itself is stable and more suitable for long-term use. When the bacterium is used, however, complete oxidation results and methanol formation is not detected as the subsequent reaction, alcohol oxidation, proceeds with some dehydrogenases contained in the bacterium. If alcohol dehydrogenase is inhibited selectively, methanol consumption will be suppressed and methanol formation will be detected.

In this communication we describe the accumulation of methanol by a cyclopropane treated bacterium. As pyrroloquinoline quinone (PQQ) dependent dehydrogenases are strongly inhibited by cyclopropanol, we anticipated the formation of cyclopropanol by the cyclopropane treated bacterium.

M. trichosporium (OB3b) was cultivated in a baffle-walled shaking flask (500 ml) with methane and air (*ca.* 2:3 in

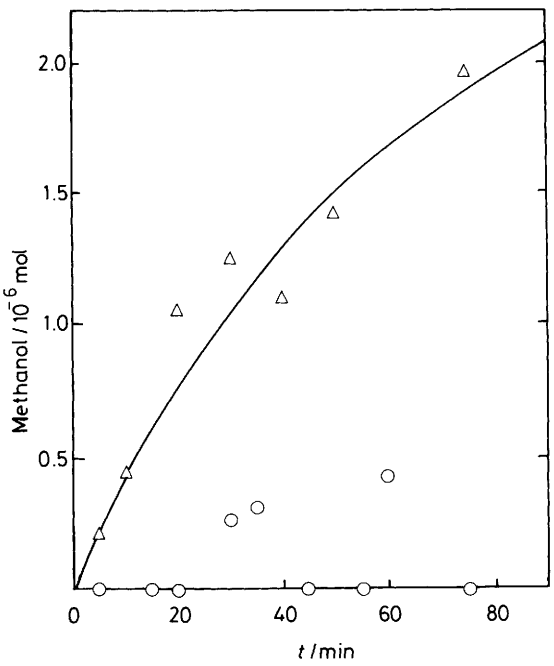

Figure 1 Time dependence of methanol formation. The sample solution (total volume 3.0 ml) contains cell suspension (0.5 ml), methane (1.0×10^{-4} mol), oxygen (8.0×10^{-5} mol), and HCO_2Na (5.0×10^{-5} mol) in phosphate buffer (3.0×10^{-2} M) (pH 7.0). The reaction was carried out at 30 °C. △: cyclopropane treated cells, ○: non-treated cells.

volume). After the cultivation the cell was harvested by the centrifugation (× 5000 g) and was resuspended in phosphate buffer (0.1 mol dm^{-3}) (pH 7.0). Cyclopropane treatment was performed as follows. Cyclopropane was bubbled in cell suspended solution for several minutes at 30 °C and then the excess cyclopropane was removed by helium bubbling. Methane oxidation was carried out in phosphate buffer (8.3×10^{-2} mol dm^{-3}) (pH 7.0) at 30 °C. In the reaction vessel (*ca.* 15 ml) cell suspension (0.5 ml, *ca.* 1 mg-dry wt.), methane (1.0×10^{-4} mol), oxygen (8.0×10^{-5} mol), and HCO_2Na (5.0×10^{-5} mol) were introduced to start the reaction. The reaction products were analysed by gas chromatography.

Figure 1 shows the time dependence of methanol formation by methane oxidation with *M. trichosporium* (OB3b). When the cells treated with cyclopropane were used, methanol was accumulated and its concentration increased with reaction time. When non-treated cells were used, methanol accumulation was not observed. The above results are explained by the selective inhibition of alcohol dehydrogenase by cyclopropane treatment. The following results support the above conclusion. When the cells were treated with cyclopropane, methanol oxidation was strongly inhibited. However, the cyclopropane treatment did not affect the methane monooxygenase activity (the activity was measured by propylene oxide formation by propene oxidation). The blocking mechanism is not yet clarified, but it is considered that cyclopropanol, formed by cyclopropane treated bacteria, combines with PQQ, the coenzyme of alcohol dehydrogenase, and inhibits the enzyme activity. The mechanism of the cyclopropane treatment inhibition is currently being investigated.

M. trichosporium (OB3b) was kindly provided by Professor Nobuo Kato of Tottori University. This work was partially supported by a Grant-in-Aid for Scientific Research on Priority Areas 'Macromolecule-Complexes (No. 63612507)' from the Ministry of Education, Science and Culture, Japan.

Received, 27th November 1989; Com. 9/05063I

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