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# Microbial Oxidation of Gaseous Hydrocarbons: Production of Secondary Alcohols from Corresponding *n*-Alkanes by Methane-Utilizing Bacteria

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Over 20 new strains of methane-utilizing bacteria were isolated from lake water and soil samples. Cell suspensions of these and of other known strains of methane-utilizing bacteria oxidized *n*-alkanes (propane, butane, pentane, hexane) to their corresponding secondary alcohols (2-propanol, 2-butanol, 2-pentanol, 2-hexanol). The product secondary alcohols accumulated extracellularly. The rate of production of secondary alcohols varied with the organism used for oxidation. The average rate of 2-propanol, 2-butanol, 2-pentanol, and 2-hexanol production was 1.5, 1.0, 0.15, and 0.08 μmol/h per 5.0 mg of protein in cell suspensions, respectively. Secondary alcohols were slowly oxidized further to the corresponding methylketones. Primary alcohols and aldehydes were also detected in low amounts (rate of production were 0.05 to 0.08 μmol/h per 5.0 mg of protein in cell suspensions) as products of *n*-alkane (propane and butane) oxidation. However, primary alcohols and aldehydes were rapidly metabolized further by cell suspensions. Methanol-grown cells of methane-utilizing bacteria did not oxidize *n*-alkanes to their corresponding secondary alcohols, indicating that the enzymatic system required for oxidation of *n*-alkanes was induced only during growth on methane. The optimal conditions for in vivo secondary alcohol formation from *n*-alkanes were investigated in *Methylosinus* sp. (CRL-15). The rate of 2-propanol and 2-butanol production was linear for the 40-min incubation period and increased directly with cell protein concentration up to 12 mg/ml. The optimal temperature and pH for the production of 2-propanol and 2-butanol were 40°C and pH 7.0. Metal-chelating agents inhibited the production of secondary alcohols. The activities for the hydroxylation of *n*-alkanes in various methylotrophic bacteria were localized in the cell-free particulate fractions precipitated by centrifugation between 10,000 and 40,000 × *g*. Both oxygen and reduced nicotinamide adenine dinucleotide were required for hydroxylation activity. The metal-chelating agents inhibited hydroxylation of *n*-alkanes by the particulate fraction, indicating the involvement of a metal-containing enzyme system in the oxidation of *n*-alkanes. The production of 2-propanol from the corresponding *n*-alkane by the particulate fraction was inhibited in the presence of methane, suggesting that the subterminal hydroxylation of *n*-alkanes may be catalyzed by methane monooxygenase.

In recent years, microbial growth on methane and methanol has been the subject of extensive investigation, especially with regard to the potential of methane- and methanol-based fermentation for single-cell protein production (4, 8, 29, 33).

Methylotrophs are microorganisms that grow nonautotrophically on compounds containing one or more carbon atoms but no carbon-carbon bonds (3). The methane-utilizing bacteria are generally obligately dependent on methane or methanol as sole sources of carbon and energy for growth (1, 5, 7, 12, 25, 28, 32). Recently, there have been reports on the isolation and characterization of bacteria in pure culture that utilize methane as well as complex organic compounds as sources of carbon and energy (19, 24, 25).

Recently we discovered that whole-cell suspensions of methane-utilizing bacteria catalyzed the oxidation of various *n*-alkanes to the corresponding alcohols. Both primary and secondary alcohols were produced from their corresponding *n*-alkanes (R. N. Patel, A. I. Laskin, and P. Derelanko, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, O17, p. 199; 21).

In this report, we describe the oxidation of *n*-alkanes to the corresponding secondary alcohols by cell suspensions of methane-utilizing bacteria

and describe the effects of various environmental factors on the production of secondary alcohols from *n*-alkanes by cell suspensions of *Methylosinus* sp. (CRL-15). We also demonstrate that the enzyme catalyzing the hydroxylation of *n*-alkanes in various methylotrophic bacteria is localized in the particulate fractions. The accompanying paper (23) describes the production of methylketones from their corresponding *n*-alkanes by cell suspensions of methylotrophic bacteria.

## MATERIALS AND METHODS

**Organisms.** *Methylosinus trichosporium* (OB3b) and *Methylobacter capsulatus* (Y) were kindly provided by R. Whittenbury (School of Biological Sciences, University of Warwick, Coventry, Warwickshire, England). Some organisms used in these studies were newly discovered strains of methane-utilizing bacteria isolated in our laboratories from soil and lake water samples by an enrichment culture technique with methane as the sole source of carbon and energy. The methane-utilizing organisms were maintained on mineral salts (7) agar plates in a desiccator under an atmosphere of methane and air (1:1, vol/vol) at 30°C.

**Preparation of cell suspensions.** Cultures of methane-utilizing organisms were grown in 300-ml flasks containing 50 ml of mineral salts medium (7) with methane (methane and air, 1:1, vol/vol) as the sole source of carbon and energy. Flasks were fitted with a rubber stopper with a glass tube and clamps for gassing. Flasks were partially evacuated and filled with a gas mixture of methane and air (1:1, vol/vol). Cultures were incubated at 30°C on a rotary shaker at 200 rpm.

Cells were harvested during exponential growth by centrifugation at 12,000 × *g* for 15 min. Cells were washed twice with 50 mM sodium phosphate buffer, pH 7.0, and the final pellet was suspended in fresh buffer.

**Preparation of cell-free particulate fractions.** Organisms were grown at 30°C in 2.8-liter flasks containing 700 ml of mineral salts medium (7) with methane (methane and air, 1:1, vol/vol) as the sole carbon and energy source. Cells were harvested during exponential growth by centrifugation at 12,000 × *g* for 15 min at 4°C. Cells were washed twice with 25 mM potassium phosphate buffer, pH 7.0, containing 5 mM $MgCl_2$. Cells were suspended in the 10 ml of the same buffer. The cell suspensions at 4°C were disintegrated by a single passage through a French pressure cell (15,000 lb/in$^2$) and centrifuged at 5,000 × *g* for 15 min to remove unbroken bacteria. The supernatant solution crude extract was then centrifuged at 40,000 × *g* for 60 min, yielding particulate [P(40)] and soluble [S(40)] fractions. The P(40) fraction was suspended in 2 ml of 25 mM potassium phosphate buffer, pH 7.0, containing 5 mM $MgCl_2$ and homogenized at 4°C.

**Assay for alcohol production.** When whole-cell suspensions were used, 0.5 ml of cell suspension was placed in 10-ml vials at 4°C which were sealed with a rubber cap. The gaseous phase of the vials was removed by vacuum and then replaced with a gas mixture of the substrates (e.g., propane or butane) and oxygen at a 1:1 (vol/vol) ratio. In the case of liquid substrates (*n*-pentane, *n*-hexane), 5 to 10 μl of the substrate was put in the vials. The vials were then incubated at 30°C on a rotary shaker at 200 rpm. The secondary alcohols (2-propanol, 2-butanol, 2-pentanol, 2-hexanol) formed were assayed by gas chromatography by using a stainless-steel column (20 ft by ⅛ inch [about 61 by 0.3 cm]) packed with 10% Carbowax 20M on 80/100 Chromosorb W (The Perkin-Elmer Corp., Norwalk, Conn.). The column temperature was maintained isothermally at 130°C, and the carrier gas flow was 35 ml of helium per min. The primary and secondary alcohol products were identified by retention time comparison and cochromatography with authentic standards. The amounts of alcohols produced were determined from the peak area, using a standard graph of amount of authentic compound (nanomoles) against the area of the peak. A 1- to 2-μl sample was removed with a syringe and was assayed directly by gas chromatography. Duplicate measurements were performed for each assay. A typical gas chromatogram with separation of authentic $C_3$ hydrocarbons (propane, 1-propanol, 2-propanol, propanal, and acetone) is shown in Fig. 1. Similarly, other *n*-alkanes, primary alcohols, secondary alcohols, aldehydes, and ketones were separated and identified by gas chromatography retention time comparison and cochromatography with authentic standards.

The oxidation of propane and butane by P(40) and S(40) fractions was measured at 30°C by estimating the production of 2-propanol and 2-butanol, respectively. The reaction mixtures contained, in 1.0 ml: 150 mM potassium phosphate buffer, pH 7.0, containing 3 mmol of $MgCl_2$, 0.6 ml; 10 μmol of reduced nicotinamide adenine dinucleotide (NADH), 50 μl; and cellular fraction.

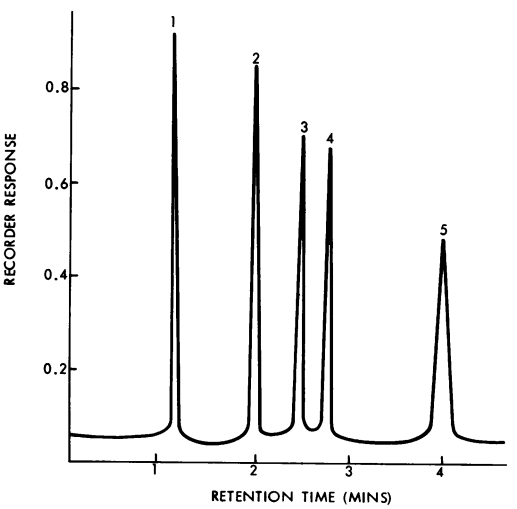


FIG. 1. *Typical gas chromatogram for authentic $C_3$ hydrocarbons. The conditions for gas chromatography were as described in the text. (1) n-Propane; (2) propanal; (3) acetone; (4) 2-propanol; (5) n-propanol.*

Reaction mixtures were contained in 10-ml vials at 4°C. Vials were sealed with rubber caps. The gaseous phase in the vials was removed by vacuum and then replaced with a gas mixture of propane or butane and oxygen (1:1, vol/vol). For liquid substrates, 10 μl of substrate was used directly. Vials were then incubated at 30°C on a rotary shaker at 200 rpm.

The production of primary and secondary alcohols was assayed by flame ionization gas chromatography by injecting 2 μl of samples directly after 5-, 10-, and 15-min incubations of the reaction mixture. Protein was determined by the method of Lowry et al. (14).

## RESULTS

**Isolation of methane-utilizing bacteria.** Enrichment and isolation techniques used were similar to those described earlier (32). Soil samples collected from various sites at the Exxon Bayway refinery and lake water samples were used as inocula. The 1-g soil samples were suspended in 10 ml of mineral salts medium and allowed to stand at room temperature for 1 h to settle soil particles. The supernatant solution was inoculated into 300-ml flasks containing 50 ml of mineral salts medium. In the case of lake water samples, 10 ml of sample was inoculated into 50 ml of salts medium. Flasks were fitted with rubber stoppers with a glass tube and clamp for gassing. Flasks were partially evacuated and refilled with a gas mixture of methane and air (1:1, vol/vol). The enrichment flasks were incubated at 30°C on a shaker at 200 rpm. Within 72 to 96 h the culture medium became turbid, presumably due to the growth of methane-utilizing bacteria.

Serial dilutions of the enrichment cultures were made and spread on mineral salts agar plates. The plates were incubated in a desiccator under an atmosphere of methane and air (1:1, vol/vol) at 30°C. Isolated colonies were picked and restreaked. Over 20 strains of methane-utilizing organisms were isolated and identified according to the scheme of Whittenbury et al. (32). Some of the newly isolated strains are shown in Table 1.

TABLE 1. *Designation of newly isolated methane-utilizing strains*

| Methane-utilizing isolate | ER&E[a] designation |
|---|---|
| *Methylosinus* sp. | CRL-15 |
| *Methylosinus* sp. | CRL-16 |
| *Methylomonas* sp. | CRL-17 |
| *Methylocystis* sp. | CRL-18 |
| *Methylobacter* sp. | CRL-19 |
| *Methylomonas* sp. | CRL-20 |
| *Methylomonas methanica* | CRL-21 |
| *Methylomonas* sp. | CRL-22 |
| *Methylobacter* sp. | CRL-23 |
| *Methylococcus capsulatus* | CRL-24 |
| *Methylobacterium* sp. | CRL-26 |
| *Methylomonas* sp. | CRL-M4P |
| *Methylomonas* sp. | CRL-M8Y |
| *Methylomonas* sp. | CRL-M6P |
| *Methylomonas* sp. | CRL-M7P |
| *Methylobacter bovis* | CRL-M1Y |
| *Methylococcus capsulatus* | CRL-M1 |
| *Methylobacter* sp. | CRL-M6 |

[a] Exxon Research & Engineering Co.

**Oxidation of *n*-alkanes by methane-utilizing bacteria.** Most methane-utilizing bacteria are obligately dependent on methane or methanol as the sole source of carbon and energy for growth. Although methane-utilizing bacteria do not grow on other *n*-alkanes (e.g., ethane, propane, butane), cell suspensions of organisms grown on methane oxidized *n*-alkanes. The products of oxidation of *n*-alkanes were identified as the corresponding alcohols. Both primary and secondary alcohols were detected as products of oxidation. However, primary alcohols were rapidly metabolized further by cell suspension and did not accumulate in high amounts. The rates of production of primary alcohols and aldehydes from oxidation of *n*-alkanes (propane and butane) were about 0.05 to 0.08 μmol/h per 5.0 mg of protein in cell suspensions. The rates of conversion of *n*-alkanes (propane and butane) to the corresponding secondary alcohols (2-propanol, 2-butanol) by cell suspensions of some newly isolated strains and known strains of Whittenbury et al. (32) are shown in Table 2. The secondary alcohols produced accumulated extracellularly. The rates of production of secondary alcohols varied among the various methane-utilizing organisms tested. There was no oxidation of *n*-alkanes observed under anaerobic conditions.

Cell suspensions of methane-utilizing bacteria grown on methanol did not catalyze the oxidation of *n*-alkanes, indicating that the enzymatic systems required for the oxidation of *n*-alkanes are induced only during growth on methane.

We selected *Methylosinus* sp. (CRL-15) to determine the effect of environmental factors that influence the production of 2-propanol and 2-butanol from oxidation of *n*-propane and *n*-butane, respectively.

**Time course of production.** The oxidation of *n*-propane and *n*-butane to 2-propanol and 2-butanol, respectively, by cell suspensions of *Methylosinus* sp. (CRL-15) was linear during the first 40 min (Fig. 2). The rate of production of 2-propanol and 2-butanol decreased upon further incubation of the reaction mixtures. The reactions were carried out in 50 mM phosphate buffer, pH 7.0, at 30°C on a rotary water bath shaker. Heat-killed cell suspensions of *Methylosinus* sp. (CR-15) did not catalyze the oxida-

TABLE 2. *Oxidation of n-alkanes to secondary alcohols by cell suspensions of methane-utilizing organisms*[a]

| Microorganism | Conversion rate (μmol/h per 5.0 mg of protein) | |
|---|---|---|
| | *n*-Propane to 2-propanol | *n*-Butane to 2-butanol |
| *Methylosinus trichosporium* (OB3b) | 2.5 | 1.5 |
| *Methylococcus capsulatus* (CRL-24) | 1.1 | 1.0 |
| *Methylobacter capsulatus* (Y) | 0.20 | 0.09 |
| *Methylosinus* sp. (CRL-15) | 2.1 | 1.2 |
| *Methylobacterium* sp. (CRL-26) | 1.4 | 0.80 |
| *Methylomonas* sp. (CRL-17) | 1.6 | 1.2 |

[a] Reactions were carried out as described in Materials and Methods. The products were identified and estimated by gas chromatography.

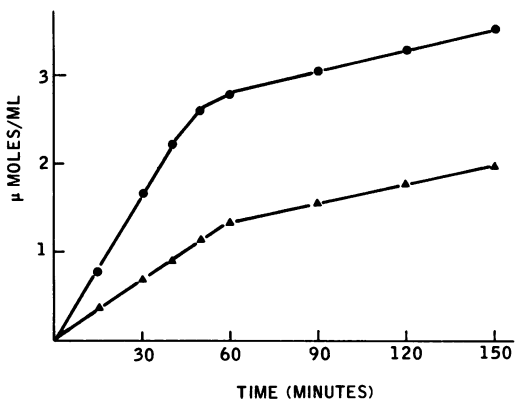


FIG. 2. *Time course of the production of 2-propanol and 2-butanol from oxidation of propane and butane, respectively, by cell suspensions (5.8 mg of protein) of Methylosinus sp. (CRL-15). Symbols:* (○) *2-propanol;* (△) *2-butanol.*

tion of *n*-alkanes to secondary alcohols, indicating an enzyme-catalyzed reaction. The secondary alcohol production was measured within 40 min whenever the effect of a variable was tested.

**Effect of cell concentration on secondary alcohol production.** The oxidation of *n*-propane and *n*-butane was dependent upon cell protein concentration. The rate of production of 2-propanol and 2-butanol was linear with cell protein concentrations from 1 to 12 mg of cell protein per ml, using suspensions of *Methylosinus* sp. (CRL-15). As cell protein concentration increased, the rate of production of 2-propanol and 2-butanol decreased (Fig. 3). The reactions were carried out in 50 mM phosphate buffer, pH 7.0, at 30°C for 30 min on a rotary water bath shaker.

**Further oxidation of secondary alcohols.** The rate of production of secondary alcohols by cell suspensions of *Methylosinus* sp. (CRL-15) decreased after a 40-min incubation of the reaction mixture (Fig. 2), suggesting, among other possibilities, that further oxidation of secondary alcohols occurred. To analyze these possibilities, 10 μmol of 2-propanol and 2-butanol was added to cell suspensions of *Methylosinus* sp. (CRL-15). Secondary alcohols slowly disappeared and were further oxidized to their corresponding methylketones.

**Effect of temperature and pH on secondary alcohol production.** The optimal temperature for the production of 2-propanol and 2-butanol by cell suspensions of *Methylosinus* sp. (CRL-15) was found to be around 40°C. Upon increasing the temperature to 45°C, the rate of production of secondary alcohols from oxidation of *n*-alkanes decreased. The optimal pH for the production of 2-propanol and 2-butanol from the oxidation of *n*-propane and *n*-butane, respectively, was found to be around 7.0 (Fig. 4).

**Substrate specificity.** The production of secondary alcohols from various *n*-alkanes was examined by using cell suspensions of *Methylosinus* sp. (CRL-15), *Methylococcus capsulatus* (CRL-24), and *Methylobacterium* sp. (CRL-26). 2-Propanol and 2-butanol were produced at higher rates than 2-pentanol and 2-hexanol (Table 3).

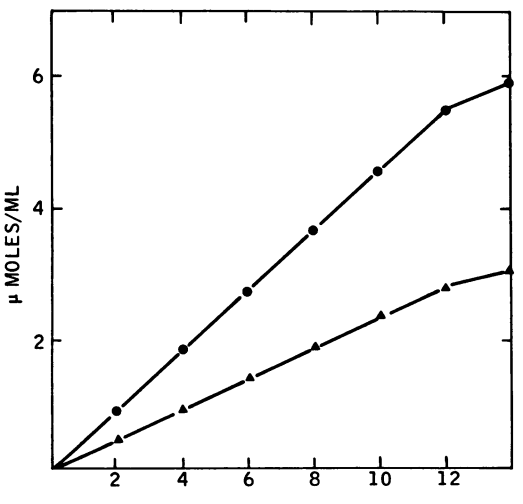


FIG. 3. *Effect of cell mass (protein) concentration on the production of 2-propanol and 2-butanol from the oxidation of propane and butane, respectively, by cell suspensions of Methylosinus sp. (CRL-15). Symbols:* (○) *2 propanol;* (△) *2-butanol.*

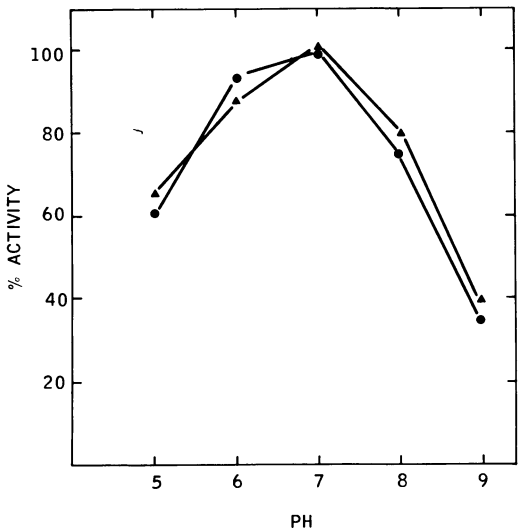


FIG. 4. *Effect of pH on the production of 2-propanol and 2-butanol from the oxidation of propane and butane, respectively, by cell suspensions (5.0 mg of protein) of Methylosinus sp. (CRL-15). Symbols: (○) 2-propanol; (△) 2-butanol. 100% activity = 2.0 and 1.0 μmol/h per mg of cell protein of 2-propanol and 2-butanol produced, respectively. The reactions were carried out at 30°C as described in Materials and Methods.*

TABLE 3. *Substrate specificity for n-alkane oxidation by methane-utilizing bacteria*[a]

| Microorganisms | Conversion rate (μmol/h per 5.0 mg of protein) | | | |
|---|---|---|---|---|
| | *n*-Propane to 2-propanol | *n*-Butane to 2-butanol | *n*-Pentane to 2-pentanol | *n*-Hexane to 2-hexanol |
| *Methylosinus* sp. (CRL-15) | 2.1 | 1.2 | 0.2 | 0.1 |
| *Methylococcus capsulatus* (CRL-24) | 1.1 | 1.0 | 0.15 | 0.08 |
| *Methylobacterium* sp. (CRL-26) | 1.4 | 0.80 | 0.08 | 0.05 |

[a] Reactions were carried out as described in Materials and Methods. The products were identified and estimated by gas chromatography.

**Oxidation of *n*-alkanes by cell-free systems.** Cell-free P(40) fractions derived from various methylotrophic organisms catalyzed the hydroxylation of *n*-alkanes. Both primary and secondary alcohols were detected as the products of oxidation of alkanes ($C_1$ to $C_4$ tested) (Table 4). The products of oxidation were identified by gas chromatography after incubating P(40) fractions with various substrates at 30°C for 10 min. The hydroxylation of *n*-alkanes was dependent upon the presence of oxygen and NADH.

We examined the effects of various metal-binding agents on the oxidation of methane to methanol (terminal hydroxylation reaction) and propane to 1-propanol and 2-propanol (terminal and subterminal hydroxylation reaction) by the P(40) fraction of *Methylosinus* sp. (CRL-15). Various inhibitors were incubated with the P(40) fraction for 10 min at 4°C. The products of the reaction were estimated by gas chromatography after 10 min of incubation at 30°C on a rotary shaker. The production of methanol, 1-propanol, and 2-propanol was inhibited by various metal-binding compounds (Table 5). This suggests the involvement of metal ion(s) in the oxidation of both methane to methanol and propane to 1-propanol and 2-propanol.

The question of whether the same enzyme was involved in the terminal hydroxylation of methane to methanol and subterminal hydroxylation of propane to 2-propanol was examined by substrate competition experiments. The experiment consisted of determining the effect of methane on the oxidation of propane to 2-propanol by the P(40) fraction of *Methylosinus* sp. (CRL-15). There was a reduction in the amount of 2-propanol formed in the presence of methane (Table 6). Hence, methane inhibited the conversion of propane to 2-propanol, presumably by competing for the available enzymatic site.

## DISCUSSION

An important phenomenon revealed by these studies is the ability of methylotrophic bacteria to metabolize substrates which they cannot utilize for growth. Thus, gaseous hydrocarbons other than methane normally do not support growth of methane-utilizing bacteria; however, they are oxidized by methylotrophs.

Leadbetter and Foster (13) first reported that washed-cell suspensions of *Pseudomonas methanica* grown on methane do not oxidize *n*-propane and *n*-butane. However, these were oxidized when present simultaneously in growth culture with methane as the growth substrate. By using this cooxidation technique, they observed the production of a series of homologous oxidation products from cosubstrate gases. Thus, *n*-propanol, propionic acid, and acetone were produced from *n*-propane; *n*-butanol, butyric acid, and 2-butanol were produced from *n*-butane.

In this report, we demonstrate the oxidation of *n*-alkanes by washed-cell suspensions of various methylotrophic bacteria. Both primary and secondary alcohols were detected as products of oxidation. However, primary alcohols were rapidly metabolized further by cell suspensions. Methane-utilizing bacteria contained soluble

TABLE 4. *Hydroxylation of n-alkanes to corresponding alcohols by P(40) fraction of methylotrophs*[a]

| Organism | Conversion rate (μmol/h per mg of protein) | | | | | |
|---|---|---|---|---|---|---|
| | Methane to methanol | Ethane to ethanol | Propane to: | | Butane to: | |
| | | | 1-Propanol | 2-Propanol | 1-Butanol | 2-Butanol |
| *Methylosinus* sp. (CRL-15) | 3.0 | 2.1 | 0.60 | 0.75 | 0.42 | 0.45 |
| *Methylococcus capsulatus* (CRL-24) | 2.4 | 1.5 | 0.45 | 0.60 | 0.35 | 0.39 |

[a] Reactions were carried out as described in Materials and Methods. The products were identified and estimated by gas chromatography.

TABLE 5. *Inhibition of oxidation of n-alkanes to corresponding alcohols in P(40) fractions of Methylosinus sp. (CRL-15)*[a]

| Inhibitor | % Inhibition | | |
|---|---|---|---|
| | Methane to methanol | Propane to: | |
| | | 1-Propanol | 2-Propanol |
| Thiosemicarbazide | 98 | 97 | 96 |
| 1,10-Phenanthroline | 100 | 100 | 100 |
| α,α-Bipyridyl | 100 | 100 | 100 |
| Potassium cyanide | 85 | 92 | 90 |
| Imidazole | 92 | 92 | 90 |
| Thiourea | 87 | 88 | 85 |

[a] Uninhibited rates of production of methanol, 1-propanol, and 2-propanol were 3.0, 0.60, and 0.75 μmol/h per mg of protein. All inhibitors were tested at 1 mM final concentration.

TABLE 6. *Effect of methane on the oxidation of propane to 2-propanol by P(40) fractions of Methylosinus sp. (CRL-15)*[a]

| Substrate | Concn of 2-propanol formed (μmol/h per mg of protein) |
|---|---|
| Propane-$O_2$ (50:50) | 0.80 |
| Propane-methane-$O_2$ (25:25:50) | 0.45 |
| Propane-helium-$O_2$ (25:25:50) | 0.80 |
| Methane-$O_2$ (50:50) | 0 |

[a] Reactions were carried out as described in Materials and Methods. The product was identified and estimated by gas chromatography.

phenazine methosulfate-linked methanol dehydrogenase with high specific activity which also catalyzed oxidation of primary alcohols (15, 16, 18). Secondary alcohols were not oxidized by methanol dehydrogenase. Cell suspensions of methylotrophic bacteria slowly catalyzed oxidation of secondary alcohols. Cell-free extracts of methylotrophic bacteria and yeasts contained an oxidized nicotinamide adenine dinucleotide-linked secondary alcohol dehydrogenase (9, 22). Oxidation of straight-chain aldehydes in methylotrophic bacteria was catalyzed by an aldehyde dehydrogenase (11, 19, 20). The ability to oxidize *n*-alkanes was observed only with cells of the methylotrophic bacteria that had been grown on methane. Methanol-grown cells did not oxidize *n*-alkanes, indicating that the enzymatic system required for oxidation of *n*-alkanes is induced only during growth on methane.

The oxidation of methane by cell suspensions of methane-utilizing bacteria (10, 17) and cell-free P(40) fractions derived from methane-utilizing bacteria (6, 27, 31) is inhibited by various metal-binding agents. We have demonstrated that the oxidation of *n*-alkanes to the corresponding secondary alcohols by the cell-free P(40) fraction of *Methylosinus* sp. (CRL-15) is also inhibited by metal-binding agents. These results suggest that the oxidation of methane to methanol and of other *n*-alkanes to secondary alcohols may be catalyzed by methane monooxygenase in all methylotrophs. The inhibition of oxidation of *n*-alkanes to the corresponding secondary alcohols by the cell-free P(40) fraction of *Methylosinus* sp. (CRL-15) in the presence of methane further supports this notion.

Colby et al. (2) reported that the soluble methane monooxygenase from *Methylococcus capsulatus* (Bath) catalyzed the NADH- and oxygen-dependent oxidation of methane to methanol. It also catalyzed the oxidation of various *n*-alkanes ($C_2$ to $C_8$). The enzyme was not a terminal hydroxylase, and both primary and secondary alcohols were produced from *n*-propane and *n*-butane. Unlike *Methylococcus capsulatus* (Bath), it was reported that the particulate fractions derived from *Methylosinus trichosporium* (OB3b) (31), *Methylococcus capsulatus* (Texas) (27), and *Methylomonas methanica* (6) catalyzed the *n*-alkane (methane, ethane, or propane)-stimulated NADH oxidation and oxygen consumption. The products of *n*-alkanes oxidation were not identified.

We have now demonstrated that the particulate fractions derived from various methane-utilizing bacteria catalyze the oxidation of *n*-alkanes to the corresponding alcohols. Both primary as well as secondary alcohols were identified as the products of oxidation of propane and butane.

Recently, Stirling et al. (30) reported that the crude extracts derived from *Methylosinus trichosporium* (OB3b) and *Methylomonas methanica* catalyzed the oxidation of various *n*-alkanes. Both primary and secondary alcohols were identified as products of oxidation of *n*-alkanes.

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