Metabolism of Propane, n-Propylamine, and Propionate by Hydrocarbon-Utilizing Bacteria

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Studies were conducted on the oxidation and assimilation of various three-carbon compounds by a gram-positive rod isolated from soil and designated strain R-22. This organism can utilize propane, propionate, or n-propylamine as sole source of carbon and energy. Respiration rates, enzyme assays, and $^{14}$CO$_2$ incorporation experiments suggest that propane is metabolized via methyl ketone formation; propionate and n-propylamine are metabolized via the methylmalonyl-succinate pathway. Isocitrate lyase activity was found in cells grown on acetate and was not present in cells grown on propionate or n-propylamine. $^{14}$CO$_2$ was incorporated into pyruvate when propionate and n-propylamine were oxidized in the presence of NaAsO$_2$, but insignificant radioactivity was found in pyruvate produced during the oxidation of propane and acetone. The n-propylamine dissimilatory mechanism was inducible in strain R-22, and amine dehydrogenase activity was detected in cells grown on n-propylamine. Radiorespimeter and $^{14}$CO$_2$ incorporation studies with several propane-utilizing organisms indicate that the methylmalonyl-succinate pathway is the predominant one for the metabolism of propionate.

Microbes have diverse mechanisms for the initial oxidative attack on hydrocarbon substrates and for the dissimilation of the oxygenated product. Two mechanisms of oxidative attack on short-chain n-alkanes (C$_3$-C$_4$) have been demonstrated. These are terminal oxidation (2, 9) and methyl ketone formation (14, 19). The terminally oxygenated product may be catabolized further by alpha oxidation (7, 21), i.e. removal of one carbon at each step, or by beta oxidation (4, 28). Methyl ketones can be metabolized to the alpha-hydroxy ketone (17, 19) as with acetone to acetal, or the methyl ketone can be subterminally oxidized to an acetate ester (8) which is cleaved to yield acetate and a primary alcohol.

Very little is known about the microbial metabolism of the various derivatives of the short-chain hydrocarbons and primary amines. The microbial metabolism of methylamine has been investigated thoroughly. Leadbetter and Gottlieb (15) showed that a gram-negative diplococcus metabolized methylamine by incorporating the methyl group of methylamine into serine by hydroxymethylation of glycine. The same pathway for the degradation of methylamine was observed for Pseudomonas sp. AM1 (12) and Pseudomonas sp. MS (10). Studies on the metabolism of higher amines by microbes are lacking. Eady and Large (5) observed that a purified amine dehydrogenase from Pseudomonas sp. AM1 showed a high affinity for the primary aliphatic monoamines from methylamine through n-hexylamine, but the metabolic pathways for degradation of these compounds were not studied.

These findings prompted a study of the metabolism of propane, propionate, and n-propylamine by hydrocarbon-utilizing bacteria.

MATERIALS AND METHODS

**Microorganisms.** The microorganism used for most of this study was strain R-22, a short, gram-positive, non-acid-fast rod of an unclassified genus tentatively identified with a group of organisms as α-methyl/glucoside positive (Ruth E. Gordon, Institute of Microbiology, Rutgers University, personal communication). This organism is capable of utilizing, as sole source of carbon and energy, all n-alkanes from C$_3$ through C$_{14}$ and an array of long-chain alkenes, ketones, alcohols, and organic acids.

Other strains used for a comparative study of propionate metabolism were as follows: Mycobacterium vaccae strain JOB5; M. album strains 7E4 and 7E1B1W; and M. rhodochrous strains OFS, A78, and 7E1C. These organisms can use propane as sole

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source of carbon and energy. All cultures, including R-22, were maintained on agar slants with propane as substrate.

**Media and growth conditions.** Organisms were cultured on the mineral salts medium of Leadbetter and Foster (13) supplemented with the appropriate carbon source. Propionate (Na) and n-propylamine were added at a concentration of 0.2%. The n-propylamine was neutralized with HCl and filter-sterilized. Propylene was added by replacing 50% of the air in a closed flask with the gaseous alkane. For growth on propane, propionate, or n-propylamine, inocula were preadapted by growth on the appropriate substrate.

**Manometric studies.** Manometric techniques were those described by Umbreit et al. (24).

**Enzyme assays.** Cell-free extracts for isocitrate lyase determinations were made by suspending the cells in tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.9) and disrupting them in a French pressure cell or by sonic disruption at 4°C for 20 min in a sonic dismembrator (Quigley-Rochester, Inc., Rochester, N.Y.). Extracts for α-ketoglutarate dehydrogenase were made in 67 mM phosphate buffer (pH 7.5). Debris was removed by centrifugation at 17,000 × g for 30 min at 4°C. Protein was assayed by the method of Lowry et al. (18).

Isocitrate lyase (three-α, isocitrate glyoxalylase, EC 4.1.3.1) was assayed by the method of Daron and Gunasalus (3) using formation of the 2,4-DNP as a control. The solvent was n-butanol-ethanol-0.5 N NH₄OH (70:10:20). The oxidation of n-propylamine by cell-free extracts of strain R-22 was determined by the method of Eady and Large (5). Activity was measured with Na-methyl-phenazonium methosulfate (phenaexitone methosulfate) as electron acceptor by following its reoxidation with 2,6-dichlorophenol-indophenol (DCPIP) spectrophotometrically at 600 nm. The assay was started by addition of n-propylamine, and the rate of decrease in absorbance at 600 nm, consequent on the reduction of DCPIP, was followed at 27°C in a Spectronic 20 colorimeter against a blank containing all reagents except DCPIP.

**14CO₂ Incorporation experiments.** The procedure of Smith and Kornberg (22) was followed with an endogenous control with and without added NaAsO₃. NaH¹⁴CO₃ (10 μCi) was added to each vessel with 50 μmoles of propionate, n-propylamine, or acetone. Gaseous substrates were added as a 50: 50, gas-air mixture. Parallel flasks with malate as substrate were run for each cell type to measure CO₂ exchange reactions. After incubation at 30°C for 150 min, carrier pyruvate was added (12.5 μmoles). The keto acids were converted to the respective 2,4-DNP by the method of El Hawary and Thompson (6). Two milliliters of 0.1% 2,4-DNP in 2 N HCl was added to each flask, and the mixture was incubated at room temperature for 30 min. The 2,4-DNP was then extracted six times with 2-ml volumes of ethyl acetate. The extracts were combined and dried under N₂. After suspension in 0.2 ml of ethyl acetate, a 10-μlter sample was spotted on Whatman no. 1 filter paper and chromatographed as described above. The developed chromatograms were air-dried and sprayed with alcoholic KOH. The spot corresponding to pyruvate was placed in a scintillation vial, and scintillation fluid was added. The radioactivity was measured in a Mark I analyzer (Nuclear-Chicago Corp., Des Plaines, Ill.). A nonradioactive pyruvate spot was used to determine background.

**RESULTS**

Manometric studies were conducted on non¬proliferating cells of strain R-22 after growth on propane, n-propylamine, and propionate. Q(O₂) values (microliters of O₂ uptake per milligram of cells per hour) were calculated for each test substrate, and the results are presented in Table 1.

| Strain R-22 cells were harvested after growth on n-propylamine, acetate, and propionate, and cell-free extracts were prepared. These extracts were assayed for isocitrate lyase activity (Table 2). When cells were grown on propionate or n-propylamine, no isocitrate lyase activity was detected, whereas cells grown on acetate had significant activity. Although cells grown on propane contained isocitrate lyase activity in most experiments, the results with this substrate were inconsistent.

Nonproliferating cells of strain R-22 grown on propane, acetone, n-propylamine, and propionate were exposed to the corresponding growth substrates in the presence of NaH¹⁴CO₃. Sodium arsenite was added to one of the duplicate flasks to effect the accumulation of pyruvate. If propane, acetone, or n-propylamine are metabolized through propionate, labeled pyruvate should also be accumulated in the presence of labeled bicarbonate and ar-
TABLE 1. Oxidation of three-carbon substrates by nonproliferating cells of strain R-22 after growth on propane, n-propylamine, and propionate

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Q(O₂)a</th>
<th>n-Propylamineb</th>
<th>Propionatec</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Propylamine</td>
<td>5</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>Propane</td>
<td>56</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>105</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>92</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>110</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Acetone</td>
<td>148</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Propionate</td>
<td>6</td>
<td>25</td>
<td>87</td>
</tr>
</tbody>
</table>

* Expressed as microliters of O₂ uptake per milligram of cells per hour.
* Cells grown in mineral salts medium in a 50:50, propane-air atmosphere were suspended in physiological saline. Each Warburg vessel contained 2.5 mg (dry weight) of cells in 2.0 ml of saline, 0.2 ml of substrate (50 μmoles) or a 50:50, gaseous substrate-air atmosphere, and 0.2 ml of 20% KOH in the center well. The incubation temperature was 30°C. Endogenous Q(O₂) values were subtracted.
* Cells grown in mineral salts medium with 0.2% neutralized substrate.

TABLE 2. Isocitrate lyase levels in cell extracts of strain R-22 after growth on acetate, n-propylamine, and propionate

<table>
<thead>
<tr>
<th>Growth substrate</th>
<th>Specific activity a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>1.3</td>
</tr>
<tr>
<td>n-Propylamine</td>
<td>0</td>
</tr>
<tr>
<td>Propionate</td>
<td>0</td>
</tr>
</tbody>
</table>

* Units per milligram of protein in which one unit is the amount of enzyme necessary for the cleavage of 1 μmole of isocitrate in 10 min at 30°C.

Discussion

Nonproliferating cells of strain R-22 grown on propane oxidized the substrates oxygenated in the C-2 position (Table 1) more readily than propane and n-propylamine, confirmed that the enzyme was absent from cells grown on propionate. The enzyme activity in cells grown on n-propylamine was 23.7 units. (1 unit = amount of enzyme necessary to reduce 1 μmole of DCPIP per minute with n-propylamine used as substrate).

Experiments were conducted on several organisms (strains OFS, 7E1C, A78, 7E4, 7E1B1W, and JOB5) that utilize propane as sole substrate to determine whether the methyld,lmalonate-succinate pathway for propionate utilization is a common pathway in propene-oxidizing microbes. Significant radioactivity was present in the pyruvate produced during propionate oxidation for all the organisms tested (Table 4). Radiorepirometer experiments were conducted with these same organisms as further confirmation of the pathway of propionate utilization. The pattern of 14C evolution from propionate-1-14C, -2-14C, and -3-14C by nonproliferating propionate-grown cells was determined. Results are presented in Fig. 2.

DISCUSSION

Nonproliferating cells of strain R-22 grown on propane oxidized the substrates oxygenated in the C-2 position (Table 1) more readily than
and hydroxylated of isocitrate JOB5. Propionate pane. suggests that found This pathway is via a
pionyl-CoA, dehydrogenated to intermediates suggests that results indicating tone, propane-grown with acetate formation. Low
ion. Earlier studies (26) pyruvate during metabolism of strain R-22 cells on propionate. Cells were suspended in tri(hydroxymethyl)aminomethane buffer (pH 7.9). Final volume was 3.5 ml and was incubated at 30 C for 150 min.

Each vessel contained 50 μmoles of substrate and 10 μCi of NaH14CO3. NaAsO2 (4 μmoles) was added to one flask for each substrate. Cells were killed by steam distillation. All vessels were incubated 150 min. 

a Values correct for background and endogenous count.

Strain R-22 cells grown on propionate oxidized 1,2-propanediol, 2-propanol, and acetone, indicating that some propionate may be metabolized via a C2-C1 split similar to that found in the metabolism of acetone or propane. Propionate could be activated to propionyl-CoA, dehydrogenated to acrylyl-CoA, and hydroxylated to lactyl-CoA. The induction of isocitrate lyase has been found when this pathway is involved in propionate metabolism. This pathway has been demonstrated in Clostridium propionicum (16), Peptostreptococcus (1, 11), and in species of Pseudomonas (25). Enzyme assays revealed that strain R-22 cells grown on propionate have no detectable isocitrate lyase activity (Table 2), whereas there is activity in cells grown on acetate. The absence of detectable isocitrate lyase suggests that the amount of propionate metabolized via acetate is of minor overall importance.

If propionate is metabolized through methylmalonyl-CoA to succinate, a carboxylation reaction occurs and can be detected by adding 14CO2 to cells metabolizing these substrates. Addition of NaAsO2, which inhibits the en-

![Graph](image-url)

Fig. 1. The induction of propionate-grown strain R-22 cells to the oxidation of n-propylamine. Each respirometer vessel contained a total volume of 2.4 ml: 2.0 ml of cells (8 mg, dry weight) diluted in mineral salts medium; 0.2 ml of 30% KOH in the center well; and 0.2 ml of substrate (50 μmoles). One vessel contained 100 μg of chloramphenicol.

![Graph](image-url)

Fig. 2. Radiorespirometric patterns for the utilization of propionate-1-14C, -2-14C, and -3-14C by nonproliferating cells of various propane-utilizing bacterial strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Malate</th>
<th>Malate + NaAsO2</th>
<th>Propionate</th>
<th>Propionate + NaAsO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFS</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>130</td>
</tr>
<tr>
<td>7E1C</td>
<td>0</td>
<td>0</td>
<td>177</td>
<td>3,710</td>
</tr>
<tr>
<td>A78</td>
<td>2</td>
<td>15</td>
<td>1,140</td>
<td>3,550</td>
</tr>
<tr>
<td>7E4</td>
<td>1</td>
<td>332</td>
<td>6</td>
<td>695</td>
</tr>
<tr>
<td>7E1B1W</td>
<td>0</td>
<td>84</td>
<td>5</td>
<td>2,500</td>
</tr>
<tr>
<td>JOB5</td>
<td>0</td>
<td>885</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Relative rate of 14C-carbon dioxide incorporation into pyruvate produced by nonproliferating cells of a number of hydrocarbon-utilizing organisms during oxidation of malate and propionate

- PROPIONATE
- N-PROPYL AMINE
- ENDOGENOUS
- CHLORAMPHENICOL ADDED

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zyme dihydrolipoyl dehydrogenase of the pyruvate dehydrogenase enzyme complex, should cause pyruvate accumulation (20). If carboxylation occurred, the pyruvate accumulated would be isotopically labeled. The production of radioactive pyruvate (Table 3) during oxidation of propionate suggests that propionate was indeed metabolized by the methylmalonyl-CoA pathway. The relatively low count was in part due to a deleterious effect of arsenite on the oxidation of propionate, but also lends support to the possible occurrence of another minor pathway for the metabolism of propionate as mentioned above. Radiospirometric studies with strain R-22 (Fig. 2) show that the rate of 14CO2 production from propionate-L-14C was higher than from propionate-2-14C or -3-14C. Radioactive CO2 from propionate-2-14C and -3-14C was evolved at essentially the same rate, which confirms that the number 2 and 3 carbons become part of a symmetrical molecule. This suggests the presence of the propionyl-CoA carboxylase–methylmalonyl-CoA pathway of propionate utilization according to Wegener et al. (29).

The methylmalonic acid pathway thus appears to be functional in the metabolism of propionate in strain R-22 as well as M. succace strain JOBS (28). Radiospirometric studies (Fig. 2) and 14CO2 incorporation into pyruvate (Table 4) for a variety of propane-utilizing strains suggest that this pathway may be the predominant one for the degradation of propionate in organisms capable of utilizing propane.

The QO2 values for n-propylamine-grown cells of strain R-22 (Table 1) indicate that terminally oxidized intermediates are involved in the oxidation of n-propylamine. Cells grown on n-propylamine did not significantly oxidize 1,2-propanediol, 2-propanol, or acetone; whereas propionate-grown cells oxidized the compounds at a greater rate. If propylamine was oxidized terminally, the reactions involving deamination, oxidation, and carboxylation of the oxidized intermediate might occur tightly bound to the enzyme; thus, all propionate formed would be converted to succinate while bound to the enzyme. Labeled pyruvate produced (Table 3) during the oxidation of n-propylamine, in the presence of 14CO2, indicates that propionate is a key intermediate and that the methylmalonyl-CoA pathway is instrumental in the metabolism of the substrate. The amount of 14CO2 incorporated into pyruvate was greater during oxidation of n-propylamine than during oxidation of propionate. This further supports the hypothesis that a greater portion of the propionate formed during oxidation of n-propylamine is metabolized by one pathway. The absence of any detectable amount of isocitrate lyase activity (Table 2) suggests that no two carbon intermediates are initially formed in the oxidation of n-propylamine.

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