The Role of the Terminal and Subterminal Oxidation Pathways in Propane Metabolism by Bacteria

By GILLIAN M. STEPHENS* AND HOWARD DALTON
Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

(Received 19 December 1985; revised 28 April 1986)

Several strains of propane-utilizing bacteria were isolated from samples of pond and river water. They could be classified into three groups according to their ability to grow on acetone, and a representative strain from each group was selected for detailed study. All three strains belonged to the genus Arthrobacter. Strain B3aP could not grow on acetone and could not oxidize acetone after growth on propane. Strain PrI03 grew slowly on acetone but could not oxidize acetone after growth on propane. Both strains excreted acetone during growth on propane. Simultaneous adaptation experiments demonstrated that, whilst propane was oxidized to both propan-1-ol and propan-2-ol, only propan-1-ol was metabolized completely suggesting that only the terminal oxidation pathway was involved in propane dissimilation. The third strain, B2, grew rapidly on acetone and was induced for acetone oxidation after growth on propane, suggesting that both propan-1-ol and propan-2-ol were produced and metabolized completely since no products of propane oxidation could be detected in culture supernatants. We compare these findings with previous reports concerning pathways of propane oxidation.

INTRODUCTION

Several studies of propane-utilizing bacteria suggest that the principal route for propane dissimilation is via acetone, i.e. by subterminal oxidation (Fig. 1; Perry, 1980). This conclusion is based upon the finding that some strains of propane-utilizing bacteria converted propane to acetone which accumulated in the culture medium (Lukins & Foster, 1963) and that bacteria grown on propane were induced for acetone oxidation (Lukins & Foster, 1963; Perry, 1968). Mycobacterium vaccae JOB5 contained isocitrate lyase after growth on propan-2-ol or propane but not after growth on propanoate or propan-1-ol (Vestal & Perry, 1969), suggesting that propane and propan-2-ol were degraded via a two-carbon intermediate. Propanoate was apparently oxidized via the methyl malonate pathway but enzymes of this pathway were not present after growth on propane, suggesting that propane was not oxidized via propanoate. However, the fatty acid profile of propane-grown cells resembled that of propanoate-grown cells in possessing a greater proportion of fatty acids containing odd numbers of carbon atoms than the fatty acid profile of cells grown on acetate, ethane or propan-2-ol (Vestal & Perry, 1971; Perry, 1980). This suggested that some terminal oxidation of propane did occur. Furthermore, M. vaccae JOB5 grown on propane oxidized propan-1-ol and propanoate rapidly (Perry, 1968), while acetone oxidation by these cells occurred at a very low rate compared with that in cells grown on propan-2-ol. It has been concluded from these and other studies that, although some terminal oxidation of propane does occur, the principal route for propane oxidation is via acetone (Perry, 1980). We consider that such a conclusion cannot be drawn from the data available since it is possible that propane was oxidized principally via propan-1-ol and propanoate and that propanoate was metabolized via a pathway other than the methyl malonate pathway.

Abbreviations: AMS, ammonium mineral salts; NAMS, nitrate/ammonium mineral salts; NMS, nitrate mineral salts.

0001-3169 © 1986 SGM
pathway. For example, operation of the malonic semialdehyde or malonic semialdehyde CoA pathways for propanoate oxidation (Wegener et al., 1968) would require the induction of isocitrate lyase. It therefore seemed possible that the terminal oxidation pathway might be more important in propane oxidation than has previously been considered.

In order to study the relative importance of the terminal and subterminal oxidation pathways in propane metabolism we isolated some propane-utilizing bacteria and investigated the pathway of propane oxidation in these strains by growth substrate specificity studies, product accumulation studies and simultaneous adaption experiments. Our isolates oxidized propane to both propan-1-ol and acetone but the majority effected complete oxidation of propane via the terminal oxidation pathway only. We discuss the significance of the partial oxidation of propane to acetone by these bacteria.
Organisms and growth. The bacteria used in this study were isolated from ethane or propane enrichment cultures of samples of pond or river water. The propane-utilizing bacteria were routinely grown in the ammonium mineral salts (AMS) medium described by Whittenbury et al. (1970), to a defined salts medium containing NH₄Cl (0.5 g l⁻¹). The bacteria were isolated in nitrate/ammonium mineral salts (NAMS) medium (AMS plus 1 g KNO₃ l⁻¹). Nitrate mineral salts (NMS) medium contained KNO₃ (1 g l⁻¹) as the sole nitrogen source. Solidified media were prepared by the addition of Difco Bacto-agar (15 g l⁻¹). All media were used at pH 6.8 and were sterilized by autoclaving at 120 °C for 15 min. Sterile phosphates were subsequently added to the cooled medium. Flammable carbon sources were considered self-sterile and were added after the medium had been inoculated. Non-flammable carbon sources, such as glucose, were added before inoculation. Gaseous substrates were added to liquid cultures (50 ml) in sealed 250 ml flasks by injecting 100 ml of the gas from a syringe through a Suba-seal. Agar plates were placed in 'Tupperware' containers which were gassed with the contents of football bladders inflated with the appropriate gaseous alkane. The concentration of gas in the containers was approximately 50% (v/v) in air. All cultures were grown at 30 °C and liquid cultures were incubated on an orbital shaker. Stock cultures of the propane-utilizing bacteria were maintained on propane–AMS agar and were subcultured at least every 2 weeks.

Isolation of gaseous-alkane-utilizing bacteria. Samples (250 μl) of pond or river water were used to inoculate 50 ml NAMS medium in a 250 ml flask. Ethane or propane (50 ml) was added as the carbon source and the cultures were incubated at 30 °C on an orbital shaker until turbidity developed, usually within 4 to 10 d. The different strains were isolated from serial dilutions of these cultures, which were spread on NAMS agar plates. Single colonies were then streaked on NAMS agar plates and single colonies from these cultures were transferred to liquid medium. Single colonies were then isolated by serial dilution and pure cultures were obtained by re-streaking on NAMS agar.

Measurement of cell density. Cell density was measured routinely as OD₅₄₀ using a Pye Unicam SP1800 spectrophotometer. Dry weights were estimated from a standard curve of OD₅₄₀ versus dry weight, where the dry weights were measured by the filtration method. Samples of cultures at different phases of growth and at a known OD were filtered by suction through dried, pre-weighed filter discs with a pore size of 0.2 μm. The collected cells were washed with nitrogen-free medium by filtration and the cells were then dried on the filters at 60 °C and subsequently weighed.

Tests of growth substrate specificity. The ability of the propane-utilizing bacteria to grow on a variety of potential growth substrates was tested in liquid culture. The accuracy of such tests depends upon the purity of the substrate and it was ensured that most were at least 99% pure. Those which were less pure were used at concentrations giving a maximum of 0.004% impurities, which should be insufficient to support growth. Cultures were incubated for up to 14 d and any that grew were checked rigorously under the microscope for contaminating microorganisms. All tests were repeated at least three times.

Preparation of cell suspensions. Cultures (200 ml) were grown on the required substrate to the late exponential phase of growth and harvested by centrifugation at 25000 g for 5 min at 4 °C. The cells were washed in 200 ml mineral salts (MS) medium, pH 6.8 (i.e. AMS medium lacking a nitrogen source) and centrifuged as before. The cell pellets were resuspended in MS medium to a final OD of 22–30 and used to assay the ability of the cells to oxidize various substrates. Experiments on any one suspension were completed within 3 h, during which time the ability to oxidize the various substrates remained stable.

Respiration studies. The ability to oxidize various substrates was tested by measuring the stimulation of O₂ uptake in suspensions of cells harvested from flask cultures. O₂ consumption was measured at 30 °C using a Clark-type O₂ electrode (Rank Brothers) and chart recorder (Bryans Southern Instruments Ltd). The dissolved O₂ concentration in air-saturated MS medium was determined by the method of Robinson & Cooper (1970). Assays for oxidation of the various substrates were done in a stirred reaction mixture containing 2.7 ml (or 2.6 ml) MS medium, 200 μl cell suspension and 100 μl (or 200 μl) substrate solution. The reaction mixture was saturated with air, the plunger was inserted and the system allowed to re-equilibrate. The cell suspension was then injected by syringe and the endogenous respiration rate was measured over 2 min. The substrate was then injected and O₂ consumption was measured. Stimulated rates of O₂ consumption were corrected for the endogenous respiration rate. Substrates which were readily soluble in water were prepared as 150 mM solutions and 100 μl was added to the assay mixture to give a 5 mM solution. Propane (200 μl) was added as a saturated solution in water prepared at 25 °C to give a final concentration of 0.095 mM (calculated from the data of McAuliffe, 1966). Assays were done at least twice and were repeated with different preparations of cells.

Gas chromatography. A GCD gas chromatograph with a flame ionization detector (Pye Unicam) was used for analysis of culture supernatants and supernatants from substrate consumption assays. Aqueous samples (5 μl) were injected through an inlet heated to 150 °C on to a 4 mm × 1.5 m glass column packed with 1:1 (w/w) mixture of Porapak Q with Porapak N. N₂ was used as the carrier gas at a flow rate of 30 ml min⁻¹; the oven was maintained at 150 °C and the detector at 200 °C. Retention times of all the possible volatile products of propane metabolism were determined to ensure that the identification was accurate. The retention times (min) were as...
follows: methanol, 1.58; propan-1-ol, 12.72; propan-2-ol, 9.26; propanal, 12.72; acetone, 8.06; methyl acetate, 6.44. Acetol was determined by raising the oven temperature to 200 °C; its retention time was 10.37 min. Propanoate, acetate and propan-1,2-diol gave extremely broad flat peaks well after the other compounds. The results of chromatographic analyses were quantified by comparison with authentic external standards. 1,2-Epoxypropane was determined in a similar fashion except that the chromatography column contained Porapak Q.

Chemicals. Porapak Q and Porapak N were obtained from Phase Separations. Propane (99.5%) was obtained from BOC and methanol (99.8%), propan-1-ol (99.8%), propan-2-ol (99.5%), propanal (95%), acetone (99.5%), 1,2-propanediol (99%) and methyl acetate (98%) from BDH. Methyl glyoxal (purity unspecified) was obtained from Sigma and acetol (purity unspecified) from Aldrich. Formaldehyde was prepared by heating paraformaldehyde in water at 100 °C for 3–4 h. All other chemicals were of the highest purity available.

RESULTS

Isolation and identification of propane-utilizing bacteria

Over 30 strains of propane-utilizing bacteria were isolated from samples of water from ponds and rivers in Warwickshire, UK. Two of these strains (B2 and B3aP) were studied in detail since they grew rapidly and did not flocculate in liquid culture; the third strain used in this investigation (PrI03) was a gift from Dr D. I. Stirling (Celanese Research Corporation, Summit, NJ, USA). All three strains were very similar. Colonies were pink when grown on propane/AMS agar plates but growth in liquid culture on propane did not result in visible pigmentation. They were obligately aerobic, Gram-positive rods; they were not acid-fast, did not form spores or contain metachromic granules. Several of their characteristics suggested that the strains were Arthrobacter species, according to the criteria of Keddie (1974). The clearest indication was the classic coccus/short rod to long rod transition which occurred as the cultures entered the exponential phase of growth, followed by a transition to cocci/short rods on entering the stationary phase.

All three strains grew fairly rapidly on gaseous alkanes after a lag phase of approximately 10 h; for example, strain B3aP grew at a rate of 0.13 h⁻¹ on ethane in AMS medium in batch culture. The strains utilized ethane, propane and butane, but not methane, for growth. Other growth substrates included acetate, glucose and nutrient broth.

Excretion of products during growth on propane

Strains B3aP, PrI03 and B2 were grown on propane in AMS medium and culture supernatants were analysed for volatile products 3 d after inoculation. Cultures of strains B3aP and PrI03 contained 2–2.5 mm acetone whilst strain B2 did not excrete acetone. No other products were detected although the method used (gas chromatography on Porapak Q/N) was suitable for the detection of propan-1-ol, propan-2-ol and propanal in addition to acetone.

Growth on potential intermediates of propane oxidation

The propane-utilizing bacteria were tested for their ability to grow on the intermediates of the two possible pathways for propane oxidation (Fig. 1). All three strains grew on propan-1-ol and propanoate. Propanal was toxic to these organisms since addition of 0.05% propanal to cultures growing on propane completely inhibited growth. However, the organisms were able to grow on propane in the presence of 0.02% propanal which also permitted rapid growth when supplied as the sole carbon source, although the final cell density was necessarily low. Evidently, all three strains possessed the metabolic potential to oxidize propane via the terminal oxidation pathway. The strains differed in their ability to utilize intermediates of the subterminal oxidation pathway. Strain B3aP could utilize neither propan-2-ol nor acetone for growth, although these substrates were not toxic at the concentration tested (0-2%) in the presence of propane. In contrast, strain B2 grew rapidly on propan-2-ol and acetone, reaching an OD₅₄₀ of approximately 1 after 2 d incubation. Strain PrI03 grew very slowly on these substrates, requiring incubation for up to 10 d. All three strains could use methyl acetate, acetol and methyl glyoxal for growth. Methanol was not utilized.
Propane metabolism by bacteria

Fig. 2. Oxidation of propan-2-ol by strain B3aP. Washed cells of propane-grown strain B3aP were suspended in 20 mM-sodium phosphate buffer, pH 7.0, to a density of 0.68 mg dry wt ml⁻¹, and preincubated at 30°C for 2 min on a gyratory shaker. Propan-2-ol (5 mM) was added to start the reaction. Samples were taken at intervals and centrifuged to remove the cells. The supernatants were stored on ice and the quantities of acetone produced (○) and propan-2-ol remaining (●) were determined by gas chromatography on Porapak Q/N.

Table 1. Ability of strain B3aP to oxidize potential intermediates of propane metabolism after growth on propane, propan-1-ol or acetate

<table>
<thead>
<tr>
<th>Assay</th>
<th>Propane</th>
<th>Propan-1-ol</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>O₂ consumption [nmol min⁻¹ (mg dry wt)⁻¹]</td>
<td>propane</td>
<td>propan-1-ol</td>
</tr>
<tr>
<td>Propane</td>
<td>58</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Propan-1-ol</td>
<td>118</td>
<td>82</td>
<td>0</td>
</tr>
<tr>
<td>Propanal</td>
<td>71</td>
<td>63</td>
<td>12</td>
</tr>
<tr>
<td>Propanoate</td>
<td>29</td>
<td>42</td>
<td>41</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>73</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Acetone</td>
<td>5</td>
<td>5</td>
<td>ND</td>
</tr>
<tr>
<td>Acetate</td>
<td>16</td>
<td>23</td>
<td>78</td>
</tr>
</tbody>
</table>

Oxidation of potential intermediates of propane metabolism by strain B3aP

Strain B3aP oxidized propan-1-ol, propan-2-ol and propanal rapidly after growth on propane (Table 1). Propanoate was oxidized relatively slowly by this strain after growth on either propane or propan-1-ol. These low rates of oxidation can probably be explained by the lack of induction of an uptake system which would be required for rapid propanoate oxidation, since propanoate would be predominantly in the dissociated form at the pH (6-8) of the assay. Acetone was not oxidized at an appreciable rate by propane-grown strain B3aP. Propan-2-ol was converted almost stoichiometrically to acetone which was excreted (Fig. 2) and therefore not used for growth. This indicates that strain B3aP could not oxidize propane via the subterminal oxidation pathway. Acetate-grown cells were unable to oxidize propane and propan-1-ol whilst propanal was oxidized very slowly (Table 1). This demonstrates that the activities seen in propane-grown cells were specifically associated with propane oxidation.

Oxidation of alk-1-enes by strain B3aP

It has been suggested that alk-1-enes might be the first intermediates of alkane oxidation although there is little experimental or theoretical evidence to support this (McKenna & Kallio,
Table 2. Oxidation of potential intermediates of propane metabolism by strain PrIO3 after growth on various substrates

Strain PrIO3 was grown on propane (50%, v/v in air), propan-1-ol, propan-2-ol or acetone (all at 0.2%) and oxidation of various substrates by suspensions of washed cells was assayed by substrate-dependent stimulation of O₂ uptake. ND, Not determined.

<table>
<thead>
<tr>
<th>Assay substrate</th>
<th>propane</th>
<th>propan-1-ol</th>
<th>propan-2-ol</th>
<th>acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propane</td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Propan-1-ol</td>
<td>70</td>
<td>66</td>
<td>69</td>
<td>9</td>
</tr>
<tr>
<td>Propanal</td>
<td>46</td>
<td>70</td>
<td>130</td>
<td>ND</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>41</td>
<td>37</td>
<td>141</td>
<td>34</td>
</tr>
<tr>
<td>Acetone</td>
<td>1</td>
<td>0</td>
<td>158</td>
<td>123</td>
</tr>
<tr>
<td>Acetol</td>
<td>17</td>
<td>19</td>
<td>165</td>
<td>136</td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>21</td>
<td>ND</td>
<td>47</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 3. Ability of strain B2 to oxidize intermediates of propane metabolism after growth on some key intermediates

Strain B2 was grown on propane (50%, v/v, in air), propan-1-ol, propan-2-ol, acetone (all at 0.2%) or acetate (0.5%) and oxidation of various substrates by suspensions of washed cells was assayed by substrate-dependent stimulation of O₂ uptake. ND, Not determined.

<table>
<thead>
<tr>
<th>Assay substrate</th>
<th>propane</th>
<th>propan-1-ol</th>
<th>propan-2-ol</th>
<th>acetone</th>
<th>acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propane</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Propan-1-ol</td>
<td>146</td>
<td>59</td>
<td>195</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Propanal</td>
<td>144</td>
<td>41</td>
<td>367</td>
<td>103</td>
<td>43</td>
</tr>
<tr>
<td>Propanoate</td>
<td>ND</td>
<td>82</td>
<td>84</td>
<td>46</td>
<td>80</td>
</tr>
<tr>
<td>Acetate</td>
<td>ND</td>
<td>26</td>
<td>14</td>
<td>83</td>
<td>92</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>142</td>
<td>38</td>
<td>229</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>Acetone</td>
<td>173</td>
<td>0</td>
<td>272</td>
<td>189</td>
<td>7</td>
</tr>
<tr>
<td>Acetol</td>
<td>86</td>
<td>32</td>
<td>492</td>
<td>184</td>
<td>57</td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>70</td>
<td>ND</td>
<td>88</td>
<td>63</td>
<td>26</td>
</tr>
</tbody>
</table>

1965). We therefore tested strain B3aP for its ability to grow on ethene, propene (both at 50%, v/v, in air), hex-1-ene and oct-1-ene (both at 0.1%). None of these supported growth. Strain B3aP was then grown on propane and tested for its ability to oxidize propene. Propene was converted to 1,2-epoxypropane; no other products could be detected.

Oxidation of potential intermediates of propane metabolism by strain PrIO3

Propane-grown strain PrIO3 resembled strain B3aP since it could oxidize propan-1-ol, propanal and propan-2-ol but not acetone (Table 2). In contrast, strain PrIO3 oxidized acetone rapidly after growth on propan-2-ol or acetone. The inability of propane-grown cells to oxidize acetone suggests that propane was not oxidized via the subterminal oxidation pathway.

Oxidation of potential intermediates of propane metabolism by strain B2

Strain B2 oxidized propane at a much lower rate than strains B3aP and PrIO3. However, propan-1-ol, propanal, propan-2-ol and acetone were oxidized rapidly by propane-grown strain B2 (Table 3). The rates of acetone oxidation were similar in propane- and acetone-grown cells. The high level of activity induced during growth on propane suggests that acetone was an intermediate of propane oxidation. It appeared that acetone was oxidized via acetol by propan-2-ol- and acetone-grown cells since these cells oxidized acetol more rapidly than methyl acetate.
Propane metabolism by bacteria

Table 4. Oxidation of C-1 compounds by propane-utilizing bacteria

Bacteria were grown on propane (50%, v/v, in air) and oxidation of various substrates by suspensions of washed cells was assayed by substrate-dependent stimulation of O₂ uptake. ND, Not determined.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>B3aP</th>
<th>PrI03</th>
<th>B2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>2</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Methanol</td>
<td>74</td>
<td>74</td>
<td>42</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>103</td>
<td>103</td>
<td>70</td>
</tr>
<tr>
<td>Formate</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

O₂ consumption [nmol min⁻¹ (mg dry wt)⁻¹] by strains:

Intriguingly, the rate of acetol oxidation was rather low in propane-grown cells although higher than the rates observed in propan-1-ol- or acetate-grown cells. Nevertheless, it is tempting to conclude that propane was oxidized via both the terminal and subterminal oxidation pathways.

Oxidation of propane by acetone-grown cells

Strains PrI03 and B2 were able to oxidize propane after growth on acetone. Such coordinate induction of propane- and acetone-metabolizing systems has been observed in other organisms (Lukins & Foster, 1963; Perry, 1968). Lukins & Foster (1963) suggested that either the acetone monooxygenase can fortuitously oxidize propane or that acetone acted as a gratuitous inducer of the propane monooxygenase. If the acetone monooxygenase was responsible for propane oxidation, both acetone- and propan-2-01-grown cells ought to oxidize propane since the acetone monooxygenase would be induced during growth on either substrate. However, strains PrI03 and B2 did not oxidize propane after growth on propan-2-01 (Tables 2 and 3). This suggests that acetone acted as a gratuitous inducer of the propane monooxygenase.

Acetone excretion as an indicator of the pathway of propane oxidation

The experiments described above suggested a possible correlation between acetone excretion and an inability to oxidize propane via the subterminal oxidation pathway. We therefore examined seven additional propane-utilizing bacteria for acetone excretion and their ability to grow on propan-2-01 (the precursor of acetone). The latter test would indicate rapidly whether the organisms had the potential to oxidize acetone. Four strains (LFWY, B4, Yh, By) excreted acetone (2.2, 4.2, 2.3 and 1.6 mM respectively) during growth on propane and none utilized propan-2-01 for growth; three (B5B, YSW, E12) did not excrete acetone and were able to grow on propan-2-01. Further experiments with one of the latter strains (B5B) showed that, like strain B2, it was able to oxidize acetone after growth on propane. However, strain E12 excreted propan-1-ol and propan-2-01 after growth on propane although it was able to utilize both propan-1-ol and propan-2-01 for growth. Further tests showed that no products were detected when strain E12 was grown on propane with KNO₃ in place of NH₄Cl as the nitrogen source. If both KNO₃ and NH₄Cl were provided, alcohol excretion was observed. This suggested that ammonia inhibited alcohol oxidation in this strain, and demonstrates that cultivation conditions can affect product formation by propane utilizers.

C-1 metabolism by propane-utilizing bacteria

Other propane-utilizing bacteria have been shown to oxidize C-1 compounds (Perry, 1968). It was therefore interesting to determine whether our isolates were capable of C-1 metabolism. Strains B3aP, PrI03 and B2 did not grow on high purity methane or methanol but were able to oxidize methanol and formaldehyde after growth on propane (Table 4). Methane was oxidized very slowly by strain B3aP and not at all by strain B2. Formate oxidation was undetectable in all three strains. It is likely that methanol and formaldehyde oxidation by these strains was due to the action of non-specific alcohol and aldehyde dehydrogenases involved in propane metabolism.
DISCUSSION

Previous studies on propane-utilizing bacteria have led to the suggestion that propane is catabolized principally via the subterminal oxidation pathway (Perry, 1980). However, several of our isolates were unable to utilize propan-2-ol for growth (which indicates that the subterminal pathway was not operative) and two of the strains (B3aP and PrI03) could not oxidize acetone after growth on propane, apparently relying on the terminal oxidation pathway as their sole means of propane catabolism.

Strains B3aP and PrI03 apparently oxidized propane to both propan-1-ol and propan-2-ol suggesting that the propane oxygenase must be non-specific with respect to the site of insertion of oxygen into the propane molecule. Monoxygenases which behave in an analogous fashion have been found in a variety of organisms, including methanotrophs (Leadbetter & Foster, 1960; Colby et al., 1977; Hou et al., 1981) and liquid n-alkane utilizers (Fredricks, 1967; Klein et al., 1968; Klein & Henning, 1969; Grossebutter et al., 1979). Interestingly, such organisms have often been found to catalyse the conversion of alk-1-enes to the 1,2-epoxyalkane (May & Abbott, 1972; Colby et al., 1977; Hou et al., 1979) like strain B3aP.

The occurrence of these non-essential reactions (see e.g. May & Abbott, 1973) in such a diversity of micro-organisms suggests that the production of 1- and 2-alkanols from alkanes and the epoxidation of alk-1-enes are dictates of the enzyme mechanism rather than specific adaptations to hydrocarbon oxidation. Certainly, in the case of strain B3aP, these reactions can confer little advantage upon the cell since the oxidation of propan-2-ol to acetone would only be of value for the production of reducing equivalents whilst the epoxidation of propene would confer no advantage since the organism could not utilize alk-1-enes or their oxidation products for growth.

Nevertheless, not all alkane monoxygenases are mechanistically similar. For example, some strains of propane utilizers convert propene to acrylic acid rather than 1,2-epoxypropane (Cerniglia et al., 1976) and, whilst methane utilizers can cooxidize n-alkanes other than methane, strains B3aP, PrI03 and B2 could not oxidize methane. Suffice it to conclude that the production of propan-2-ol from propane by strains B3aP and PrI03 had very little obvious benefit.

Although propane was oxidized to both propan-1-ol and propan-2-ol by our propane-utilizing isolates some strains (B2 and B5B) were capable of complete oxidation of propan-2-ol whilst others were not. Strain PrI03 was capable of slow growth on propan-2-ol but did not oxidize propan-2-ol beyond the level of acetone during growth on propane although the necessary enzyme complement for both terminal and subterminal oxidation of propane was present. Growth was much more rapid on intermediates of the terminal oxidation pathway than on those of the subterminal oxidation pathway, suggesting that the latter pathway was rather inefficient. Enzymes of the subterminal oxidation pathway were induced during growth on propan-2-ol or acetone suggesting that both compounds could induce enzymes of the pathway. Although acetone was produced during growth on propane, the enzymes necessary for acetone metabolism were not expressed suggesting that acetone metabolism was repressed.

Strains B2 and B5B were able to grow on and oxidize intermediates of both the terminal and subterminal oxidation pathways. That the ability to oxidize the intermediates of these pathways was induced during growth of strain B2 on propane but not on acetate suggests that both pathways were involved in the dissimilation of propane. This could be proved if it could be shown that the propane monoxygenase is capable of converting propane to both propan-1-ol and propan-2-ol in vitro. Unfortunately the organisms were extremely resistant to breakage by both physical and chemical methods. A pressure drop from 276 MPa in a French pressure cell, sonication for a total of 6.5 min in the presence of ground glass or pretreatment of the cells with cycloserine (500 μg ml⁻¹) plus bacitracin (500 μg ml⁻¹) followed by lysozyme (2.5 mg ml⁻¹) treatment for 1 h and sonication proved to be the only effective methods of lysing the cells. However, these methods proved too extreme for the recovery of the relevant enzyme activities.

Lukins & Foster (1963) found that Mycobacterium smegmatis 422 accumulated acetone during growth on propane although it was able to oxidize acetone rapidly, suggesting that acetone was an intermediate of propane oxidation. In contrast, Mycobacterium vaccae JOB5 oxidized acetone
and did not excrete acetone (Perry, 1968; Ooyama & Foster, 1965) which is similar to the observations we made with strains B5B and B2. This apparent anomaly can readily be explained by the observation that cultivation conditions had a marked effect upon the pattern of product accumulation by strain E12. We suggest that *M. smegmatis* 422 excreted acetone because it was maintained under nitrogen limitation during the course of the experiment. Lower concentrations of acetone were detected in culture supernatants after growth on propane, and this might reflect another nutrient limitation occurring on approaching the stationary phase. Although we found that acetone excretion correlated with an inability of our strains to oxidize acetone, our findings with strain E12 and those of Lukins & Foster (1963) clearly demonstrate that product excretion studies can be used to draw opposite conclusions. Product excretion seems to depend both upon the organism and upon cultivation conditions and therefore cannot serve as a reliable indication of the pathway of propane oxidation.

In conclusion, it was evident that propane was metabolized exclusively via the terminal oxidation pathway by the majority of our isolates. This indicates that the terminal oxidation pathway is more important in propane dissimilation than has previously been supposed. The relative importance of the terminal and subterminal oxidation pathways in organisms expressing enzymes associated with both pathways remains obscure. The isolation of propane monooxygenase from such an organism would enable the measurement of the relative carbon flow along each of the pathways. Such a study awaits the isolation of organisms which reflect another nutrient limitation occurring on approaching the stationary phase. Although we are more amenable to the preparation of cell-free extracts.

Fiset and col. (1979) found that acetone excretion correlated with an inability of our strains to oxidize acetone, our observations we made with strains A and B2. This apparent anomaly can readily be explained by the observation that cultivation conditions had a marked effect upon the pattern of product accumulation by strain E12. We suggest that *M. smegmatis* 422 excreted acetone because it was maintained under nitrogen limitation during the course of the experiment. Lower concentrations of acetone were detected in culture supernatants after growth on propane, and this might reflect another nutrient limitation occurring on approaching the stationary phase. Although we found that acetone excretion correlated with an inability of our strains to oxidize acetone, our findings with strain E12 and those of Lukins & Foster (1963) clearly demonstrate that product excretion studies can be used to draw opposite conclusions. Product excretion seems to depend both upon the organism and upon cultivation conditions and therefore cannot serve as a reliable indication of the pathway of propane oxidation.

In conclusion, it was evident that propane was metabolized exclusively via the terminal oxidation pathway by the majority of our isolates. This indicates that the terminal oxidation pathway is more important in propane dissimilation than has previously been supposed. The relative importance of the terminal and subterminal oxidation pathways in organisms expressing enzymes associated with both pathways remains obscure. The isolation of propane monooxygenase from such an organism would enable the measurement of the relative proportions of propan-1-ol and propan-2-ol produced from propane and thus would indicate the relative importance of the terminal and subterminal oxidation pathways in organisms.

Fiset and col. (1979) found that acetone excretion correlated with an inability of our strains to oxidize acetone, our observations we made with strains A and B2. This apparent anomaly can readily be explained by the observation that cultivation conditions had a marked effect upon the pattern of product accumulation by strain E12. We suggest that *M. smegmatis* 422 excreted acetone because it was maintained under nitrogen limitation during the course of the experiment. Lower concentrations of acetone were detected in culture supernatants after growth on propane, and this might reflect another nutrient limitation occurring on approaching the stationary phase. Although we found that acetone excretion correlated with an inability of our strains to oxidize acetone, our findings with strain E12 and those of Lukins & Foster (1963) clearly demonstrate that product excretion studies can be used to draw opposite conclusions. Product excretion seems to depend both upon the organism and upon cultivation conditions and therefore cannot serve as a reliable indication of the pathway of propane oxidation.

In conclusion, it was evident that propane was metabolized exclusively via the terminal oxidation pathway by the majority of our isolates. This indicates that the terminal oxidation pathway is more important in propane dissimilation than has previously been supposed. The relative importance of the terminal and subterminal oxidation pathways in organisms expressing enzymes associated with both pathways remains obscure. The isolation of propane monooxygenase from such an organism would enable the measurement of the relative proportions of propan-1-ol and propan-2-ol produced from propane and thus would indicate the carbon flow along each of the pathways. Such a study awaits the isolation of organisms which are more amenable to the preparation of cell-free extracts.

G.M.S. is grateful to the Science and Engineering Research Council and British Petroleum Ltd for financial support.

**REFERENCES**


