Acetol monooxygenase from *Mycobacterium* Py1 cleaves acetol into acetate and formaldehyde

(*Mycobacterium* sp.; 1,2-propanediol; acetol monooxygenase)

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Received 21 April 1986
Accepted after revision 13 May 1986

1. SUMMARY

A novel acetol monooxygenase has been detected in *Mycobacterium* Py1. Extracts of 1,2-propanediol-grown cells oxidized acetol in an oxygen- and NADPH-consuming reaction. The initial oxidation product is probably hydroxymethyleneacetate, which spontaneously rearranges to acetate and formaldehyde. Acetol oxidation was inhibited by carbon monoxide, but not by 10 mM cyanide, indicating that a cytochrome P-450 type oxygenase might be involved. The enzyme activity was only detected in 1,2-propanediol- or acetol-grown cells, suggesting that this acetol monooxygenase plays a role in the metabolism of 1,2-propanediol by *Mycobacterium* Py1.

2. INTRODUCTION

Acetol (1-hydroxyacetone) is a supposed intermediate in the metabolism of propane, 1,2-propanediol and acetone by certain bacteria. Two pathways have been proposed for the metabolism of acetol. Either oxidation via methylglxal to pyruvate [1] or a carbon–carbon cleavage-involving pathway, leading to the formation of acetate and a C1-compound. For instance, in experiments with *Bacillus pyocyaneus* acetate and formate were detected as a result of acetone consumption [2], whereas formaldehyde could be trapped during growth of *Fusarium* on isopropanol, acetone and 1,2-propanediol [3]. Furthermore, isocitrate lyase activity was induced in *Mycobacterium* JOB-5 during growth on propane, indicating that propane is metabolized via acetate. Propane degradation by this organism was suggested to proceed via isopropanol and acetone to acetol, followed by a cleavage reaction yielding a C2 and C1 unit [4]. The C1 unit is probably formaldehyde [5]. However, experimental evidence for the occurrence of an enzyme catalysing the supposed carbon–carbon cleavage reaction is as yet lacking. We describe here the presence of an enzyme in *Mycobacterium* Py1 which would allow metabolism of acetol via acetate and formaldehyde.

3. MATERIALS AND METHODS

3.1. Organism and growth conditions

The isolation and characterisation of *Mycobacterium* Py1 has been described previously [6]. The organism was maintained on yeast extract (3 g/l) glucose (5 g/l) slopes (12 g/l Oxoid agar No. 3). Cells were grown in 5-l Erlenmeyer flasks contain-
ing 1 l mineral medium [7] with 0.25% (w/v) carbon source at 30°C and shaking at 1 Hz. Acetol was sterile and was added aseptically to the sterile mineral medium. Cells were harvested in the late log phase of growth by centrifugation at 16000 × g for 10 min at 4°C and washed with 50 mM potassium phosphate buffer pH 7.0. After resuspension in 5–7 ml of the same buffer, the washed cells were stored at -18°C.

3.2. Preparation of cell-free extract

Routinely, extracts were prepared from cultures grown with 0.25% (w/v) 1,2-propanediol as carbon source because 1,2-propanediol could be added to the medium prior to sterilisation. Washed cell suspensions (5–7 ml) were disrupted by ultrasonication with a Branson B-12 sonifier for 10 × 15 s with a power input of 10 W at 0°C. Whole cells and cell debris were removed by centrifugation at 27000 × g for 20 min at 4°C, and the supernatant was used as crude cell-free extract. Crude extracts could be stored for several weeks at -18°C without any activity loss. Dialysis of crude extract was performed using a Sephadex G-25 column and eluting with 50 mM potassium phosphate buffer pH 7.0. Ultracentrifugation of crude extract was performed at 100 000 × g for 1 h at 4°C.

3.3. Acetol monooxygenase assay

Acetol monooxygenase activity was measured as the initial rate of acetol-dependent increase in oxygen consumption by cell-free extract. The oxygen consumption was measured polarographically with a Clark type oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH) at 30°C. The incubation mixture consisted of 3.7 ml 0.1 M potassium phosphate buffer pH 8.0, 0.1 ml 8 mM NADPH and 0.1 ml cell-free extract. After following the acetol-independent oxygen consumption for 5 min, 0.1 ml of 80 mM acetol was added and the initial increase in the oxygen consumption rate was recorded. Measurement of NADPH consumption at 340 nm as a result of acetol addition resulted in slightly higher specific activities. This is ascribed to the fact that crude extract also contains NADPH-dependent acetol reductase. Acetol monooxygenase activity was linear with the protein concentration present in the assay system (0.06–0.3 mg/ml) and was completely destroyed after boiling for 10 min.

3.4. Chemical estimations

Acetol, acetate and methylglyoxal were measured with a Varian Aerograph series 2400 gas chromatograph fitted with a Chromosorb 101 (80–100 mesh) column. The column temperature was 190°C and the carrier gas N₂ saturated with formal acid. Acetaldehyde was determined as described previously [8]. Formaldehyde was determined with 2,5-pentadione [9] and its identity was confirmed using formaldehyde dehydrogenase (EC 1.2.1.46) from Pseudomonas putida. Protein was measured by the Lowry method, with bovine serum albumin as the standard [10].

3.5. Materials

Acetol, methylglyoxal and acetoin were from Janssen Chimica, Beerse, Belgium. 1,2-propanediol, acetone and cyclohexanone were from E. Merck Darmstadt, F.R.G. 2-hydroxycyclohexanone was from Koch-Light, Haverhill, U.K., and 2,5-pentadione was obtained from Fluka, Buchs, Switzerland. NADPH was from Boehringer, Mannheim, F.R.G. and Sephadex G-25 was from Pharmacia Fine Chemicals, Uppsala, Sweden. Formaldehyde stock-solution was prepared by heating paraformaldehyde (Lamers and Indemans, 's-Hertogenbosch, The Netherlands) in water in a closed ampoule overnight. Formaldehyde dehydrogenase was from Sigma, St. Louis, MO, U.S.A.

4. RESULTS AND DISCUSSION

Cultures of Mycobacterium Pyl growing on 1,2-propanediol transiently accumulated acetol indicating that 1,2-propanediol was metabolized by this organism via acetol. Further evidence for the involvement of acetol as the first intermediate in 1,2-propanediol metabolism was obtained from the fact that in cell-free extracts of 1,2-propanediol-grown cells a NAD⁺-dependent 1,2-propanediol dehydrogenase activity could be measured (data not shown). This enzyme oxidised 1,2-propanediol to acetol. No NAD⁺⁺- or NADP⁺⁺-dependent acetol dehydrogenase activity could be de-
tected in extracts of acetol- or 1,2-propanediol-grown *Mycobacterium* Py1 cells indicating that acetol degradation in *Mycobacterium* Py1 differs from acetol degradation in strain A1 [1]. Instead, it was possible to detect an increase in NADPH-dependent oxygen consumption by cell-free extracts of both 1,2-propanediol and acetol-grown cells upon the addition of acetol. This observation suggested that an acetol monooxygenase was present in 1,2-propanediol or acetol-grown *Mycobacterium* Py1. Dialysis of the crude extract did not result in any loss of the specific activity of the acetol monooxygenase. Substituting 1,2-propanediol for acetol in the assay system with and without NADPH did not result in a consumption of oxygen, thus eliminating the possibility that the observed oxygen consumption as a result of acetol addition was caused by the combined activities of a NADPH dependent acetol reductase and a 1,2-propanediol oxidase [11]. After ultracentrifugation acetol monooxygenase activity was fully recovered from the supernatant, indicating the enzyme is soluble.

Until now only acetol monooxygenase from rat liver has been described. The reaction product with this enzyme was methylglyoxal [12]. This implicates that rat liver acetol monooxygenase hydroxylates acetol at the C1 position, resulting in an unstable compound which spontaneously rearranges to methylglyoxal and water. Methylglyoxal could not be detected as the oxidation product of acetol in the acetol monooxygenase catalyzed reaction in extracts from *Mycobacterium* Py1.

However, several bacterial NADPH-dependent monooxygenases (EC 1.14.13.22) have been described which, in a Baeyer-Villiger type of reaction, form 1-oxa-2-oxo-cycloheptanone from cyclohexanone [13,14]. Interestingly, 2-hydroxycyclohexanone is also a substrate for these monooxygenases, resulting in the unstable compound 1-oxa-2-oxo-7-hydroxycycloheptanone which spontaneously rearranges to 6-oxohexanoate [15] (Fig. 1A). A similar reaction can be envisaged with acetol as substrate resulting in the formation of hydroxymethyleneacetate, which would spontaneously rearrange to acetate and formaldehyde (Fig. 1B). Incubation of crude extract with acetol

![Diagram A](https://example.com/diagram1.png)

![Diagram B](https://example.com/diagram2.png)

**Fig. 1.** (A) Oxidation of 2-hydroxycyclohexanone to 6-oxohexanoate by cyclohexanone monoxygenase (EC 1.14.13.22). (B) Cleavage of acetol monooxygenase via the unstable intermediate hydroxymethylene acetate.

**Table 1**

Products of acetol monooxygenase activity

The complete reaction mixture consisted of 1 ml cell-free extract (7 mg protein), 8 μmol acetol and 5 μmol NADPH in 0.1 M potassium phosphate buffer, pH 8.0, in a total volume of 2 ml, in Hungate tubes incubated at 30°C under vigorous shaking. After 10 min the reaction was stopped by adding 0.1 ml 4N HCl. After 5 min the contents of the tubes were neutralised with 4N NaOH and denatured protein was removed by centrifugation. The supernatant was analysed for acetol, acetate and formaldehyde.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Acetol consumption (μmol)</th>
<th>Acetate formation (μmol)</th>
<th>Formaldehyde formation (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>4.2</td>
<td>2.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Minus O2</td>
<td>0.5</td>
<td>&lt; 0.2</td>
<td>0</td>
</tr>
<tr>
<td>Minus NADPH</td>
<td>0.3</td>
<td>&lt; 0.2</td>
<td>0</td>
</tr>
<tr>
<td>Minus acetol</td>
<td></td>
<td>&lt; 0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Flushed with N₂ for 10 min at 0°C.

**Table 2**

NADPH-dependent oxidation of different substrates by crude extracts of 1,2-propanediol-grown *Mycobacterium* Py1

Conditions as described in MATERIALS AND METHODS for acetol monooxygenase assay. The oxygen consumption in the absence of added carbon substrate was 12 nmol min⁻¹·mg protein⁻¹.

<table>
<thead>
<tr>
<th>Substrate (2 mM)</th>
<th>Increase in oxygen consumption rate (nmol·ml⁻¹·mg protein⁻¹) +1 mM CuSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetol</td>
<td>57 &lt; 2</td>
</tr>
<tr>
<td>Acetone</td>
<td>&lt; 2 –</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>&lt; 2 –</td>
</tr>
<tr>
<td>Acetoïn</td>
<td>53 &lt; 2</td>
</tr>
<tr>
<td>2-Hydroxycyclohexanone</td>
<td>17 &lt; 2</td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>&lt; 2 –</td>
</tr>
</tbody>
</table>

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and NADPH did indeed yield acetate and formaldehyde as reaction products (Table 1). No product formation was detected in the absence of NADPH or oxygen. The slight acetol consumption in the absence of oxygen and without added NADPH is ascribed to the acetol reductase activity present in the crude extract. The lower value for formaldehyde formation in comparison with acetate formation can be accounted for by the formaldehyde dehydrogenase activity present in the extract. In a separate experiment it was shown that acetate formation (2.1 μmol) and oxygen consumption (2.07 μmol) were equimolar. Using acetoin (2-oxo-3-hydroxybutane) as substrate, acetate and acetaldehyde were detected as reaction products. Extracts from 1,2-propanediol grown Mycobacterium Py1 also show a NADPH-dependent oxygen consumption upon 2-hydroxycyclohexanone addition, which like the acetol monoxygenase activity was absent in acetate grown cells. Cyclohexanone was not oxidised (Table 2). In all cases the enzymatic oxidation could be completely inhibited by adding 1 mM CuSO₄ to the assay mixture. Future studies with purified enzyme may give a definite answer whether or not these activities are due to a single enzyme. If so, the acetol monoxygenase described here has a different substrate specificity as compared to the previously described monoxygenases which catalyse a Bayer-Villiger type of reaction [13,14].

The acetol monoxygenase and acetone monoxygenase activities in rat liver are suggested to be catalyzed by the same P-450 isoenzyme [16]. Although the enzyme of Mycobacterium Py1 clearly differs in its reaction mechanism from the rat liver acetol monoxygenase, the enzyme described here may also belong to the cytochrome P-450 type of monoxygenases [17]. The monoxygenase activity that we measured was completely inhibited after flushing with carbon monoxide and by the addition of 1 mM Cu²⁺, but 10 mM cyanide was without effect.

Acetol monoxygenase activity was only detected in 1,2-propanediol and acetol-grown cells of Mycobacterium Py1. In acetate grown cells no acetol monoxygenase activity could be measured. This strongly suggests that the monoxygenase may play a role in 1,2-propanediol metabolism by this organism. This is in line with the observation that 1,2-propanediol and acetol-grown Mycobacterium Py1 cells contained elevated levels of isocitrate lyase (EC 4.1.3.1.). Thus acetol monoxygenase can provide the acetate for synthesis of cell constituents via the glyoxylate cycle during growth of Mycobacterium Py1 on 1,2-propanediol. Therefore the possibility should be considered that this acetol monoxygenase is also present in other bacteria which assimilate certain C₃ compounds via the glyoxylate cycle as, for example, Mycobacterium JOB-5 during growth on acetone [4,5].

ACKNOWLEDGEMENT

We would like to thank dr. J.P. van Dijken for stimulating discussions and for performing the formaldehyde determinations.

REFERENCES