Oxidation of Methylmalonate Semialdehyde to Propionyl Coenzyme A in Pseudomonas aeruginosa Grown on Valine*

(Received for publication, October 26, 1967)

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SUMMARY

The conversion of methylmalonate semialdehyde to propionyl coenzyme A has been investigated with enzyme preparations made from Pseudomonas aeruginosa grown on valine. A protein fraction has been obtained which catalyzes the oxidation of methylmalonate semialdehyde, acetaldehyde, and propionaldehyde with NAD as the electron acceptor. The product of methylmalonate semialdehyde oxidation in the presence of CoA is heat stable, reacts with hydroxylamine to form propionohydroxamate, is eluted from a chromatographic column of Dowex 1 in the same position as propionyl-CoA, and therefore, appears to be propionyl-CoA. The product of propionaldehyde oxidation in the presence of CoA also has been characterized as propionyl-CoA since it is heat stable and reacts with hydroxylamine to form propionohydroxamate. Oxidation of methylmalonate semialdehyde and propionaldehyde is accompanied by the formation of equal amounts of NADH and an active acyl compound, the latter estimated as the hydroxamate. When CoA is omitted from the reaction mixture, high levels of mercaptoethanol will restore enzyme activity, but catalytic activity is altered. In the presence of CoA, the pH activity curve for the oxidation of methylmalonate semialdehyde is much broader and the specific activity of the enzyme is higher than when CoA is absent. Omission of CoA from reaction mixtures has little effect on the pH activity curve for the oxidation of propionaldehyde however. Other enzymes concerned with propionyl-CoA metabolism which have been identified in extracts of P. aeruginosa are phosphotransacetylase, propionyl-CoA carboxylase, and lactyl-CoA dehydrase.

It has been known for several years that oxidation of valine by animal tissues leads to the formation of propionate from the isopropyl carbons of valine. Peterson, Jones, and White (1) found that rats which were fed L-valine-1-13C produced glycogen labeled in positions 1, 2, 5, and 6 of glucose. This labeling pattern was identical with that obtained by Lorber et al. (2) when they fed propionate labeled in position 2 or 3 to rats, and it led Peterson et al. to propose that the 3-carbon fragment produced from valine was propionate. Kinnory, Takeda, and Greenberg (3) used rat liver homogenates to oxidize DL-valine-4,4'-14C and recovered labeled 2-ketoisovalerate, isobutyrate, 3-hydroxyisobutyrate, and propionate from the reaction mixture. Propionate was found to be labeled exclusively in carbons 1 and 3, which confirmed its origin in the isopropyl carbons of valine. Robinson et al. (4) and Robinson and Coon (5) subsequently demonstrated the following reactions of valine catabolism using liver, heart, and kidney enzyme preparations:

\[
\text{Isobutyryl-CoA} \rightarrow \text{methacrylyl-CoA} + 2\text{H}
\]

\[
\text{Methacrylyl-CoA} + \text{H}_2\text{O} \rightarrow 3\text{-hydroxyisobutyryl-CoA}
\]

\[
3\text{-Hydroxyisobutyryl-CoA} + \text{H}_2\text{O} \rightarrow 3\text{-hydroxyisobutyrate} + \text{CoA}
\]

\[
\text{NAD} + 3\text{-hydroxyisobutyrate} \rightarrow \text{methylmalonate semialdehyde} + \text{NADH}
\]

To our knowledge, there have been no published reports concerning the enzymic conversion of methylmalonate semialdehyde to propionyl-CoA, however, Kinnory et al. (3) suggested that this might occur by decarboxylation of methylmalonate semialdehyde to propionaldehyde and carbon dioxide. Atchley (6) had already shown that propionaldehyde was readily oxidized by kidney homogenates, and the pathway of propionate metabolism in animal tissues is now well known (7).

Recent studies from our laboratory provided evidence that Pseudomonas aeruginosa grown on valine converted the isopropyl carbons of valine to propionate (8). When P. aeruginosa was grown on DL-valine-4,4'-14C as the sole source of carbon, alanine from the crude protein fraction was found to be labeled in carbon atoms 1 and 3. This finding was interpreted to mean that P. aeruginosa oxidized valine by way of the animal pathway to propionyl-CoA, which was then oxidized via acryl-CoA to pro-
vide the carbon skeleton for alanine biosynthesis. The labeling pattern of aspartate was more complex; however, it could be explained in part by C-3 + C-1 condensations such as the carboxylation of propionyl-CoA and pyruvate. Studies with cell-free preparations of *P. aeruginosa* revealed that an enzyme which catalyzed the oxidative deamination of δ-valine and other amino acids was induced by growth on L-valine, and that L-valine was deaminated by transamination with 2-ketoglutarate.

The studies reported in this communication concern the pathway for the oxidation of methylmalonate semialdehyde in *P. aeruginosa* grown on valine. An enzyme fraction has been obtained which catalyzes the oxidation of methylmalonate semialdehyde to propionyl-CoA. The enzyme also catalyzes the oxidation of acetaldehyde and propionaldehyde, the latter compound being oxidized to propionyl-CoA.

**EXPERIMENTAL PROCEDURE**

**Methods**

*Cultural Methods*—*P. aeruginosa* was grown in synthetic medium with δ-valine as the sole carbon and energy source by the procedures described in a previous publication (9). The cells were harvested from 15-liter batches of medium and stored at −15° until needed. Enzyme could be recovered from the cells for several months after harvest.

*Enzymic Methods*—The standard assay used to measure activity of enzyme fractions was made to a volume of 1.0 ml containing 100 μmoles of Tris buffer, pH 9.2, 100 μmoles of mercaptoethanol, 1 μmole of NAD, 5 μmoles of methylmalonate semialdehyde or 25 μmoles of propionaldehyde, and enzyme. All components except substrate were added and the cuvette carrier was placed in the cell compartment of a Beckman model DU spectrophotometer equipped with thermostoakers, through which water was circulated at 37°. The reaction was initiated by the addition of the substrate and the increase in absorbance recorded. One unit of enzyme activity was defined as 1 μmole of NADH formed per min and specific activity was defined as units per mg of protein. Protein was determined by the method of Waddell (10).

For the preparation of the enzyme, 10 g of frozen cells of *P. aeruginosa* were brought into suspension by mixing with 30 ml of 0.05 M phosphate buffer, pH 7.0, which contained 0.02 M mercaptoethanol and 10 mg of crude pancreatic deoxyribonuclease obtained from Sigma. Addition of deoxyribonuclease facilitated suspension of the organism, which required stirring at room temperature for 20 to 30 min. The suspension was treated with sonic oscillation for 16 min using a Raytheon model DF 101 10-kC sonic oscillator. The sonic extract was centrifuged for 15 min at 16,000 × g in a Sorvall RC-2 refrigerated centrifuge. The supernatant liquid was decanted and 0.26 g of ammonium sulfate for 30 min in a water bath at 37°. The reaction was stopped by the addition of 0.1 ml of fresh neutral hydroxylamine and the absorbance at 340 μM recorded. Addition of hydroxylamine stopped the reduction of NAD immediately and the optical density was stable for several hours. A peak of enzyme activity for both methylmalonate semialdehyde and propionaldehyde was located in the region of tube 45.

The assay for the detection of phosphotransacetylase in *P. aeruginosa* was based on the procedure of Stadtman (11). The assay conditions described by Flavin and Ochoa (12) were used to detect propionyl-CoA carboxylase. The method of Baldwin, Wood, and Emery (13) was used to assay for the presence of lactyl-CoA dehydrase. The assay was done in the laboratory of Dr. W. A. Wood at Michigan State University and all auxiliary enzymes and reagents were kindly provided by Dr. Wood. Crystalline phosphotransacetylase used in assays of enzyme fractions for the presence of CoA was purchased from Calbiochem.

*Chemical Methods*—The procedure of Lipmann and Tuttle (14) was used for the determination of active acyl groups as their hydroxamic acids with the volume reduced to 1.0 ml. The standard used in this reaction was crystalline acetohydroxamate which was dissolved in 1.0 M neutral hydroxylamine. The spectrum of this material under the conditions of the Lipmann-Tuttle assay revealed a peak in the region of 300 to 320 μM with an absorbance of 0.73 optical density units per μmole of acetohydroxamate at 500 μM. This figure was used to calculate the concentration of propionohydroxamate as well. Mercaptans were determined by the method of Grunert and Phillips (15).

*Reagents*—NAD and CoA were purchased from Sigma. Acetoxyhydroxamate, propionohydroxamate, and isobutyrohydroxamate were prepared from ethyl esters of the acids by the procedure of Wise and Brandt (16). Methylmalononomono hydroxamate was prepared according to the procedure of Flavin and Ochoa (12). Diethyl acetyl phosphate was purchased from Calbiochem, whereas lithium propionyl phosphate was prepared according to procedure B of Stadtman (Reference 17, p. 228). Methylmalonate semialdehyde was prepared according to the procedure of Kupecz and Coon (18) and standardized by titration with standard base. Coenzyme A esters were prepared from their anhydrides by the procedures outlined by Stadtman (Reference 17, p. 931).

*Chromatographic Methods: Characterization of Propionyl-CoA as Product of Methylmalonate Semialdehyde and Propionaldehyde Oxidation*—The reaction mixture used for this purpose contained 1000 μmoles of phosphate buffer, pH 8.0, 100 μmoles of mercaptoethanol, 10 μmoles of NAD, 10 μg of CoA, 0.89 mg of protein, and 54 μmoles of methylmalonate semialdehyde or 500 μmoles of propionaldehyde in a volume of 10 ml. A duplicate reaction mixture was prepared which contained boiled enzyme. The reaction was started by the addition of substrate and incubated at 37° until the increase in absorbance at 340 μM was very slow, at which time a total of 3.2 μmoles of NADH had been formed. The reaction was terminated by the addition of 0.4 ml of neutral hydroxylamine which was allowed to react at room temperature for 30 min. At this time 50 ml of 0.5% ethyl alcohol were added and the white precipitate which resulted was removed by filtration. The solution was taken to dryness with the aid of a rotary evaporator with the water bath set at 50°, and the residue was taken up in 25 ml of absolute ethyl alcohol. A suspension resulted which was filtered, the filtrate was again
strate. The standard assay was used in this experiment with chromatography (Fraction 3 in Table I).

Purification

The rate of NADH formation in the oxidation of methylmalonate semialdehyde was directly proportional to the amount of purified enzyme used up to 20 μmoles of NADH formed per min in the standard assay (Fig. 1). Similar results were obtained when propionaldehyde was the substrate for the purified enzyme. The standard assay was also used to measure the activity of unfractionated extracts toward methylmalonate semialdehyde and propionaldehyde, provided the rate of NADH formation was less than 10 μmoles per min. However, as pointed out in the following paragraph, it appears that the activity obtained with crude extracts was low when methylmalonate semialdehyde was the substrate.

The results of a typical purification procedure are shown in Table I. The specific activity of the enzyme in the sonicate with methylmalonate semialdehyde as the substrate varied from 0.015 to 0.025 μ mole of NADH formed per min per mg of protein; with propionaldehyde as the substrate, the specific activity was 0.050 to 0.075. Enzyme Purification — The rate of NADH formation in the oxidation of methylmalonate semialdehyde was directly proportional to the amount of purified enzyme used up to 20 μmoles of NADH formed per min in the standard assay (Fig. 1). Similar results were obtained when propionaldehyde was the substrate for the purified enzyme. The standard assay was also used to measure the activity of unfractionated extracts toward methylmalonate semialdehyde and propionaldehyde, provided the rate of NADH formation was less than 10 μmoles per min. However, as pointed out in the following paragraph, it appears that the activity obtained with crude extracts was low when methylmalonate semialdehyde was the substrate.

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The over-all increase in specific activity in the preparation reported in Table I was approximately 32-fold with methylmalonate semialdehyde as the substrate and 16-fold with propionaldehyde. However, because of the increase in units during purification, the true increase in specific activity with methylmalonate semialdehyde as the substrate was probably closer to 20-fold. The highest specific activities obtained were about

![Figure 1. Rate of NADH formation as a function of enzyme concentration with methylmalonate semialdehyde as the substrate.](http://www.jbc.org/)
twice those shown in Table I. Crude enzyme preparations di-
played a lag in the formation of NADH with methylmalonate semifaldehyde as the substrate, the length of the lag being in-
versely proportional to the amount of enzyme present.

**Substrate Specificity**—The purified enzyme catalyzed the oxida-
tion of acetaldehyde, propionaldehyde, and methylmalonate semifaldehyde in the ratios of approximately 1.0:0.5:0.25 as
determined by the following formation of NADH in the standard assay. Barely detectable rates of NADH formation were ob-
tained with butyraldehyde, iso-butyraldehyde, and glyceralde-
hyde-3-phosphate.

**Product of Methylmalonate Semialdehyde and Propionaldehyde Oxidation in Presence of CoA**—An active acyl compound, which was detected by its conversion to a hydroxamic acid, was pro-
duced in the oxidation of methylmalonate semifaldehyde and propionaldehyde. Somewhat higher yields were obtained in the
assay with CoA, and since this was considered to be the natural
acyl acceptor, the characterization of the product was made with CoA included in the assay. The acyl acyl compound pro-
duced in the oxidation of methylmalonate semifaldehyde was heat stable as would be expected of an ester of CoA (22). In order to
demonstrate heat stability, a reaction mixture was incubated un-
til the increase in optical density was negligible, the pH was ad-
justed to 4.5, and half of the reaction mixture was heated in a
boiling water bath for 3 min. The concentration of active acyl
compound as determined by the hydroxamic acid method was
found to be 0.23 μmole per ml in the unheated material and 0.20
μmole per ml in the heated material.

The hydroxamic acid formed when neutral hydroxylamine was
added at the termination of the reaction was identified as pro-
pi onohydroxamate by three different chromatographic methods.
In the first method, the reaction was stopped by the addition of
neutral hydroxylamine and the product worked up and chroma-
tagraphed as described under “Methods.” The hydroxamic acid
formed from the product of methylmalonate semifaldehyde oxidation cochromatographed with synthetic propionohydrox-
amate.

The method used by Yamada and Jakoby (20) in their study of the oxidation of malonate semifaldehyde to acetyl-CoA by an
enzyme from *Pseudomonas fluorescens* was the second method
used to identify propionohydroxamate. A substance which re-
acted with the nitroprusside reagent and alkali was detected on
the chromatogram of the reaction mixture with native enzyme,
but was not present on chromatograms of reaction mixtures con-
taining enzyme denatured by heat. The product, assumed to be
a thiol ester, was eluted from the paper, converted to the hy-
droxamic acid, and chromatographed with the pyridine-isooamyl
alcohol solvent (20). The hydroxamic acid migrated at the same
Rₜ as propionohydroxamate.

The third method used to characterize the product of the re-
action involved chromatography of the reaction mixture over
columns of Dowex 1 as described by Kaziro and Ochoa (21).
Chromatography of a reaction mixture with boiled enzyme
yielded a single peak at tube 16, while a reaction mixture with
active enzyme produced two peaks, one at tube 16 and another
at tube 20. The tubes which comprised the second peak were
pooled and worked up as described by Kaziro and Ochoa, con-
verted to the hydroxamic acid, and chromatographed as out-
lined under “Methods.” In this case also, the hydroxamic acid
migrated on paper at the same rate as did propionohydroxamate.

No evidence was obtained for the production of methylmalono-
monohydroxamate by any of the methods used. The properties
of the active acyl compound formed in the oxidation of methyl-
malonate semifaldehyde are in agreement with its being prop-
ionyl-CoA, namely heat stability, absorption at 201 mμ, reac-
tion with the nitroprusside reagent and alkali, and conversion to
propionohydroxamate.

In the case of propionaldehyde oxidation in the presence of
CoA, the active acyl compound was also heat stable at pH 4.5
and was converted to propionohydroxamate which was identified
by the first method outlined in the previous paragraph.

**Stoichiometry between formation of NADH and Hydroxamate in Oxidation of Methylmalonate Semialdehyde and Propionaldehyde**

<table>
<thead>
<tr>
<th>Substrate and protocol</th>
<th>NADH</th>
<th>Hydroxamate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylmalonate Semialdehyde</td>
<td>0.27</td>
<td>0.31</td>
</tr>
<tr>
<td>Omit NAD</td>
<td>0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Omit CoA</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Propionaldehyde</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Omit NAD</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>Omit CoA</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Omit propionaldehyde</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Omit propionaldehyde</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The complete reaction mixture contained 5 μmoles of dithio-
thenitol, 0.5 μg of CoA, 1 amole of NAD, 10 amoles of Tris buffer, pH 8.0, 20 μg of enzyme, and either 5 amoles of methylmalonate
semialdehyde or 25 amoles of propionaldehyde in a volume of 1.0
ml. The reaction was followed by recording the optical density
change at 340 mμ. When the increase in absorbance was negli-
gible, the reaction was terminated by the addition of 200 amoles
of neutral hydroxylamine and the concentration of hydroxamic acid
was determined.

**Effect of CoA and Thiols on Reaction**—Enzyme preparations
which were dialyzed to free them of mercaptoethanol were inac-
tive with either methylmalonate semifaldehyde or propionalde-
hyde, but activity could be restored by the addition of mer-
in the presence of a high level of mercaptoethanol and in the presence of a low level of mercaptoethanol with CoA. The reaction mixtures were made to a volume of 3.0 ml and contained 300 μmoles of Tris buffer at the approximate pH values indicated, 300 rmoles of mercaptoethanol or 15 rmoles of mercaptoethanol plus 1.5 mg of CoA, 3 μmoles of NAD, 13 μmoles of methylmalonic semialdehyde or 75 μmoles of propionaldehyde, and 21 μg of enzyme. The pH was measured immediately after the completion of the reaction and this value was recorded as the pH of the test.

When the reaction mixture contained a low level of mercaptoethanol with CoA, the pH activity curve for methylmalonate semialdehyde oxidation in the presence of low thiol and CoA was only slightly changed, the main difference being a small decrease in specific activity.

A plot of enzyme activity as a function of the concentration of CoA resulted in typical substrate saturation curves except that some activity was obtained in the absence of added CoA (Fig. 3). CoA could not be detected by use of the phosphotransacetylase assay (11) in enzyme preparations obtained from the DEAE-cellulose column nor did treatment of the enzyme with Dowex 1 (22) affect its ability to catalyze the reduction of NAD when the standard assay was used.

Other Enzymes Concerned with Propionyl-CoA Metabolism in P. aeruginosa—Phosphotransacetylase (acetyl-CoA : orthophosphate acetyl transferase (EC 2.3.1.8)) was detected in sonic extracts of cells of P. aeruginosa grown on either valine or glucose and hence appears to be a constitutive enzyme. Since reports of phosphotransacetylase in strict aerobes are rare, the reaction catalyzed by the enzyme from P. aeruginosa was investigated in order to compare it to the enzyme from anaerobes and facultative bacteria. Sonic extracts of cells grown on glucose were treated with Dowex 1 to free them of CoA and then brought to 0.43 saturation with solid ammonium sulfate. The precipitate was dissolved in 0.05 M phosphate buffer, pH 7.0, and this fraction was used for the studies reported here. The data in Table III reveal that this fraction was able to catalyze the arsenolysis of acetyl phosphate and propionyl phosphate, and that arsenolysis was dependent on CoA, enzyme, and arsenate. The enzyme preparation also catalyzed the arsenolysis of acetyl-CoA and propionyl-CoA, but not of butyryl-CoA or isobutyryl-CoA, and therefore resembles the clostridial enzyme with respect to substrate specificity (Reference 17, p. 228, and Reference 22).

Since the enzyme was reasonably stable in the absence of added thiol reagents, it was possible to obtain evidence for the conversion of acyl-CoA to acyl phosphate and free CoA by measuring the increase in total sulfhydryl groups and the decrease in heat-stable active acyl compound (Table IV). When either acetyl-CoA or propionyl-CoA was incubated with the enzyme preparation, the amount of active acyl compound present at the end of the incubation period was the same as that present initially.
tially, but there was an increase in free sulfhydryl groups. When the reaction mixture was heated to destroy acyl phosphate, the decrease in total active acyl residues matched the increase in free sulfhydryl groups corresponding to the release of CoA (Table IV). Acetohydroxamate and propionohydroxamate were the only hydroxamic acids detected on paper chromatograms of reaction mixtures containing acetyl-CoA or propionyl-CoA.

Propionyl-CoA carboxylase (propionyl-CoA:carbon dioxide ligase (ADP) (EC 6.4.1.3)) was another enzyme of propionate metabolism which was identified in extracts of cells grown on valine. The data in Table V show that fixation of \(^{14}\)CO\(_2\) into a stable compound by sonic extracts of \(P\). aeruginosa required propionyl-CoA, ATP, and enzyme. Reaction mixtures were treated with alkali to hydrolyze the thiol ester linkage, and chromatographed with the isomyl alcohol-4.0 M formic acid solvent of Flavin and Ochoa (12). Under these conditions succinate was the only radioactive acid identified; presumably methylmalonyl-CoA was converted to succinyl-CoA by the unfractionated extracts. Sonic extracts made from cells of \(P\). aeruginosa grown on acetate did not catalyze the fixation of \(^{14}\)CO\(_2\), which was dependent on the presence of propionyl-CoA.

The studies reported in this communication establish that an enzyme preparation made from \(P\). aeruginosa catalyzes the oxidation of methylmalonate semialdehyde to propionyl-CoA in \(P\). aeruginosa grown on valine. \(P\. aeruginosa\) grown on acetate did not catalyze the fixation of \(^{14}\)CO\(_2\), which was dependent on the presence of propionyl-CoA.

The reaction mixtures were made to a volume of 1.0 ml and contained 50 \(\mu\)moles of potassium phosphate buffer, pH 7.0, 2 \(\mu\)moles of sodium cyanide, acetyl-CoA as indicated, and 0.73 mg of enzyme protein. After incubation for 10 min at 37\(^\circ\), 200 \(\mu\)moles of neutral hydroxylamine were added to one set of duplicate reaction mixtures while the other set was heated in a boiling water bath for 5 min before addition of hydroxylamine. Since phosphate interfered with the hydroxamic acid determination, 0.1 ml of 1.5 M calcium chloride was added and the precipitate of calcium phosphate was removed by centrifugation. Thereafter, the concentration of hydroxamic acid and mercaptan was determined as indicated under "Methods."

### Table IV

 Conversion of acetyl-CoA and propionyl-CoA to acyl phosphates and mercaptan by enzyme fraction from \(P\). aeruginosa

<table>
<thead>
<tr>
<th>Acyl-CoA present in test system</th>
<th>Acyl phosphate</th>
<th>Mercaptan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA, 1.0 (\mu)mole</td>
<td>0.44 (\mu)mole</td>
<td>0.49</td>
</tr>
<tr>
<td>Propionyl-CoA, 0.83 (\mu)mole</td>
<td>0.30 (\mu)mole</td>
<td>0.30</td>
</tr>
</tbody>
</table>

### Table V

Fixation of \(^{14}\)CO\(_2\) dependent on propionyl-CoA by extracts of \(P\). aeruginosa

<table>
<thead>
<tr>
<th>Test system</th>
<th>Radioactivity fixed (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>9000</td>
</tr>
<tr>
<td>Omit propionyl-CoA</td>
<td>2200</td>
</tr>
<tr>
<td>Omit MgCl(_2)</td>
<td>3100</td>
</tr>
<tr>
<td>Omit ATP</td>
<td>1780</td>
</tr>
<tr>
<td>Omit glutathione</td>
<td>9760</td>
</tr>
</tbody>
</table>

**Fig. 4. Metabolism of methylmalonate semialdehyde and propionyl-CoA in \(P\). aeruginosa grown on valine.**

### Discussion

The studies reported in this communication establish that an enzyme preparation made from \(P\). aeruginosa catalyzes the oxidation of methylmalonate semialdehyde to propionyl-CoA (Fig. 4). This reaction fits the interpretation placed on the labeling studies of Peterson et al. (1) and Kinney et al. (3) with rats, and those from our own laboratory with \(P\). aeruginosa (8), namely, that propionate was derived from the isopropyl carbon atoms of valine. In \(P\). aeruginosa, propionyl-CoA can be converted to propionyl phosphate by phosphotransacetylase, to succinyl-CoA, to be oxidized by way of the tricarboxylic acid cycle, or to pyruvate for alanine biosynthesis apparently by way of acrylyl-CoA (Fig. 4). It should be emphasized, however, that the direct oxidation of propionyl-CoA to acrylyl-CoA has not been demonstrated in enzyme preparations made from \(P\). aeruginosa. It is of interest that evidence for the hydration of acrylyl-CoA to lactyl-CoA was first provided by Vagelos, Karl, and Stadtman (24) with an enzyme preparation from a soil
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pseudomonad grown on propionate. Subsequently Baldwin et al. (13) purified the enzyme from Peptostreptococcus elsdenii, an anaerobe which ferments lactate to propionate without randomization of carbon atoms 2 and 3.

Two possible reaction mechanisms would account for the oxidation of methylmalonate semialdehyde to propionyl-CoA. In

\[
\text{Methylmalonate semialdehyde} + \text{NAD} + \text{CoA} + \text{Methylmalonate semialdehyde} \rightarrow \text{propionaldehyde} + \text{CO}_2 \quad (1)
\]

Pseudomonas aeruginosa grown on propionate. Subsequently Baldwin purified the enzyme without the formation of any free intermediates. Reaction 1, methylmalonate semialdehyde to propionyl-CoA, is converted to propionyl-CoA with the formation of any free intermediates such as propionaldehyde and carbon dioxide by a nonoxidative decarboxylation. Reaction 2, the direct oxidative reaction. When the concentration of propionaldehyde is decreased to a level comparable to that of methylmalonate semialdehyde, the rate of NADH formation is considerably less than that obtained with methylmalonate semialdehyde. It is possible that propionaldehyde is an intermediate, but is very tightly bound to the enzyme.

At present it is not possible to decide whether there is a common pathway for propionate catabolism in aerobic bacteria. Smith and Kornberg (26) recently demonstrated the presence of propionyl-CoA carboxylase in Micrococcus denitrificans grown aerobically on propionate. They also showed that extracts of M. denitrificans converted radioactive bicarbonate plus propionyl-CoA mainly to succinate with lesser amounts of methylmalonate, malate, and other dicarboxylic acids. Smith and Kornberg also showed that whole cells converted unlabeled propionate and labeled bicarbonate to labeled pyruvate. This piece of evidence supports their contention that the mammalian pathway of propionate metabolism was the main pathway in M. denitrificans and that the acrylyl-CoA pathway was of little consequence. On the other hand Callely and Lloyd (27) detected all of the enzymes of a pathway of propionate oxidation through acrylyl-CoA and leading to acetyl-CoA plus carbon dioxide in a colorless alga, Prototheca zopfii. Callely and Lloyd found that fortified extracts of P. zopfii oxidized propionate-2-14C to produce radioactive intermediates of the tricarboxylic acid cycle but that the only radioactive compound recovered after oxidation of propionate-1-14C was 3-hydroxypropionate. This evidence agreed with their hypothesis that the carboxyl carbon of propionate was lost during oxidation. We interpret the presence of propionyl-CoA carboxylase in P. aeruginosa grown on propionate to mean that this organism can oxidize propionyl-CoA by way of the mammalian pathway (Fig. 4). In addition, the finding of laetyl-CoA dehydrase in conjunction with the studies of the labeling pattern of alanine in cells grown on radioactive valine or propionate (8) must mean that propionate can be oxidized at positions 2 and 3 to yield pyruvate without the intermediate formation of a dicarboxylic acid. Thus it appears that P. aeruginosa contains two pathways for the oxidation of propionate, the mammalian pathway and the pathway through acrylyl-CoA. It is possible that the mammalian pathway is the main pathway for propionate catabolism while the acrylate pathway serves the function of providing 3-carbon intermediates for the formation of alanine.

**Acknowledgments**—We would like to thank M. J. Coon for several valuable discussions and for reading the manuscript before submission. We would also like to thank W. A. Wood and D. L. Schneider for their assistance in the assays of lactyl-CoA dehydrase and for their hospitality.

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