The Isolation, Purification, and Properties of Methylmalonyl Racemase*

S. H. G. Allen, R. Kellermeyer, Rune Stjernholm, Birgit Jacobson, and Harland G. Wood

From the Department of Biochemistry, Western Reserve University School of Medicine, Cleveland 6, Ohio

(Received for publication, January 25, 1963)

Methylmalonyl isomerase catalyzes the interconversion of succinyl-CoA and methylmalonyl-CoA. Mazumder et al. (8, 9), Allen et al. (10), and Overath, Kellerman, and Lynen (11) have recently demonstrated that the preparations of the isomerase contained a second enzyme, methylmalonyl racemase, which catalyzes the interconversion of the two isomeric forms termed methylmalonyl-CoA (a) and methylmalonyl-CoA (b). This reaction is an essential step in the formation of propionyl from propionate by propionic acid bacteria as illustrated below.

\[
\text{Propionyl-CoA} + \text{succinate} \xrightarrow{\text{CoA transferase}} \text{propionate} + \text{CoA}
\]

\[
\text{Sum: } 4\text{H} + \text{pyruvate} \xrightarrow{\text{propionyl kinase}} \text{propionate} + \text{H}_2\text{O}
\]

All the above enzymes have been shown to be present in propionibacteria. This paper presents a method for the isolation, purification, and assay of methylmalonyl racemase together with some of the properties of this enzyme.

EXPERIMENTAL PROCEDURE

Propionibacterium shermanii, 52W, was grown at 30° in 75 liters of a glucose-yeast extract medium as previously described (12); 250 g (wet weight) of cells were obtained.

Steps I and II Preparation of the Cell Extract and DEAE-cellulose Batch Elution—The preparation of the cell-free extract and the preliminary purification of the protein were similar to the procedures employed in Steps I and II used for the purification of methylmalonyl-oxaloacetic transcarboxylase (13). The cell-free extract (500 ml) was diluted 6-fold with cold distilled water and mixed with 2200 ml of gravity-packed DEAE-cellulose (Brown Co., 0.8 meq per g). Initial purification of the racemase was obtained by stepwise elution of the adsorbed material with potassium phosphate buffer, pH 6.8 (Table I). The 0.3 M phosphate eluate contained approximately 70% of the enzyme which had a specific activity 1.5 times that of the original preparation. Approximately 20% of the activity was found in the eluate with 0.1 M buffer and no activity could be detected in the other fractions. The protein in the 0.3 M buffer was precipitated with ammonium sulfate (0.9 saturation at 0°), and the resulting precipitate was sedimented at 23,000 × g at 4°. The preparation contained methylmalonyl-oxaloacetic transcarboxylase, methylmalonyl isomerase, and racemase, as well as other enzymes present in the propionibacteria.

Step III. Chromatography on Cellulose Phosphate—The preparation from Step II, which contained 7.7 g of protein, was diluted to 94 ml with 0.05 M phosphate buffer, pH 7.4. This protein suspension was dialyzed against 2000 ml of 0.1 M phosphate buffer, pH 7.4, for 3 hours, with a change of buffer after each hour of dialysis. The concentration of sulfate ion was then measured with a Barnstead purity meter and found to be equivalent to 1.2 M ammonium sulfate. The preparation was diluted to 1850 ml with cold distilled water to reduce the sulfate ion concentration to less than 0.08 M and then placed on a cellulose phosphate (Brown Co., 0.8 meq per g) column (5.3 × 26 cm) equilibrated with 0.5 M phosphate buffer, pH 6.8. All the protein was adsorbed and the initial effluent was discarded. The column was then washed with 0.05 M phosphate buffer; and 3.8 g of protein (III-A, Table I) were collected in the first 2900 ml. This fraction, which contained only 4% of the racemase adsorbed onto the column, was used for the isolation of malic dehydrogenase, propionyl kinase, phosphotransacetylase, CoA transferase, and methylmalonyl isomerase. Both methylmalonyl racemase and methylmalonyl-oxaloacetic transcarboxylase were adsorbed on the cellulose. The column was washed with an additional 600 ml of 0.05 M phosphate buffer, pH 6.8. The racemase was then eluted from the column with 0.15 M phosphate buffer, pH 6.8. The first 160 ml of solution contained little protein. The racemase was eluted as a single protein peak in about 875 ml, with a maximal specific activity of 15.4, and all fractions were combined into two portions. III-D, Table I, which contained enzyme of lower specific activity, and III-C, the fractions of higher specific activity. Practically no methylmalonyl-oxaloacetic transcarboxylase was present in these fractions and 3,179 units of racemase were recovered. This represented a recovery of only 18% of the racemase adsorbed onto the column. The transcarboxylase, III-D, Table I, which had a maximal specific activity of 17.3, was eluted from the column.

* This work was assisted by grants from the National Institutes of Health (E 1085 and 2G-35) of the U. S. Public Health Service.

1 The abbreviation used is: DBC, 5,6-dimethylbenzimidazolylcobamide. This was a generous gift from Dr. Karl Folkers of the Merck, Sharp and Dohme Company.

2 Manuscript in preparation.
Table I

Purification of methylmalonyl racemase from propionibacteria

<table>
<thead>
<tr>
<th>Step</th>
<th>Recovery</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Total units</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Crude extract</td>
<td>100</td>
<td>15.8</td>
<td>1.49</td>
<td>23,600</td>
<td>100</td>
</tr>
<tr>
<td>II. 0.3 M buffer DEAE-cellulose</td>
<td>72</td>
<td>7.7</td>
<td>2.2</td>
<td>16,050</td>
<td>72</td>
</tr>
<tr>
<td>III. Cellulose phosphate column</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>725</td>
<td>3.8</td>
<td>0.19</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>B, Fractions 7-18 and 27-33</td>
<td>1,555</td>
<td>0.248</td>
<td>6.4</td>
<td>1,555</td>
<td></td>
</tr>
<tr>
<td>C, Fractions 19-26</td>
<td>1,592</td>
<td>0.149</td>
<td>10.7</td>
<td>1,592</td>
<td></td>
</tr>
<tr>
<td>D, Fractions 32-65</td>
<td>32</td>
<td>0.320</td>
<td>0.1</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Sum of B-D</td>
<td>3,179</td>
<td>3,179</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV. Ammonium sulfate fractionation of III-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, 0-0.60 saturation</td>
<td>33</td>
<td>0.068</td>
<td>0.38</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>B, 0.60-0.75 saturation</td>
<td>150</td>
<td>0.068</td>
<td>19.2</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>C, 0.75-0.90 saturation</td>
<td>604</td>
<td>0.019</td>
<td>33.4</td>
<td>604</td>
<td></td>
</tr>
</tbody>
</table>

* Cells, 251 g wet weight, from 75 liters of medium were used in this preparation.
† Specific activity is expressed as micromoles of product formed per minute per milligram of protein. Total units is specific activity times total mg of protein in the fraction.

Step IV. Ammonium Sulfate Fractionation—The protein in III-C, from Step III was precipitated with 0.9 saturation ammonium sulfate and sedimented at 23,000 × g at 4°C. The preparation, which contained 129 mg of protein, was diluted to 11 ml with 0.1 M phosphate buffer, pH 7.4, and the ammonium sulfate concentration estimated as before. The solution was brought to 0.6 saturation with saturated ammonium sulfate, and the resulting precipitate sedimented at 32,000 × g. The precipitate contained 33 units of racemase with a specific activity of 0.4 and 5 units of transcarboxylase with a specific activity of 0.06 (IV-A, Table I). The fraction obtained between 0.6 and 0.75 saturation contained 7.8 mg of protein and 150 units of racemase with a specific activity of 19.2. The protein which precipitated from 0.75 to 0.90 saturation contained 18.1 mg of protein and 604 units of racemase with a specific activity of 33.4. The 0.60 to 0.75 and the 0.75 to 0.90 fractions contained no demonstrable transcarboxylase activity.

A sedimentation analysis in a Spinco model E ultracentrifuge was made on the 0.75 to 0.90 fraction (IV-C, Table I) and the protein appeared to be homogenous with a $s_{20,w} = 2.98$ S (Fig. 1A). Two other preparations of racemase which had specific activities of 31 and 36 have also been shown to be homogenous by sedimentation and to have $s_{20,w}$ values of approximately 3.

Electrophoretic analysis of the racemase was conducted in a Spinco model H electrophoresis apparatus. The protein peak migrated as a single boundary at pH 7.4 with very little asymmetry. Typical patterns are shown in Fig. 1B. The electrophoretic mobility was $8.4 \times 10^{-5}$ cm$^2$/V·sec at 3.0 volts/cm at pH 7.4.$^4$

Assay of Methylmalonyl Racemase—The racemase was assayed spectrophotometrically in the crude extract as well as during purification, by means of a series of coupled enzymatic reactions.

Acetyl-CoA + succinate $\rightarrow$ CoA + acetyl-CoA (1)

Succinyl-CoA $\rightarrow$ succinate + acetate (1)

Methylmalonyl-CoA $\rightarrow$ succinyl-CoA + acetate (1)

Methylmalonyl-CoA (b) racemase, methylmalonyl-CoA (a) (3)

Methylmalonyl-CoA (a) $\rightarrow$ pyruvate + transcarboxylase (4)

Propropionyl-CoA + oxaloacetate $\rightarrow$ propionyl-CoA + oxaloacetate (4)

Malate + DPN$^+$ $\rightarrow$ oxaloacetate + DPNH + H$^+$ + malic dehydrogenase (5)

FIG. 1. Sedimentation and electrophoretic patterns of purified methylmalonyl racemase.

A. Sedimentation patterns. The racemase, Step IV (C) (specific activity = 33.4, 7.7 mg of protein per ml) was dialyzed overnight against 0.05 M potassium phosphate buffer, pH 7.4. Sedimentation in the direction of the arrow in a Spinco model E ultracentrifuge at 60,000 r.p.m. The schlieren photographs were taken at 32 and 192 minutes. The temperature was set for 5°C. The sedimentation value was corrected for temperature as well as for the viscosity of the buffer, $s_{20,w} = 2.98$ S.

B. Electrophoretic patterns. The racemase, Step IV (C) (specific activity 33.4) was dialyzed against 0.10 M potassium phosphate buffer, pH 7.4, for 13 hours. The final protein concentration was 12.3 mg per ml. Electrophoresis was in a Spinco model H apparatus for 187 minutes at 3.0 volts per cm. Electrophoretic mobility ($\mu$) = $8.4 \times 10^{-5}$ cm$^2$/V·sec per second per volt.

Note added May 1, 1963: The molecular weight has been determined to be 29,000 ± 2,700 by the method of Archibald, assuming a partial specific volume of 0.76.
In the assay the methylmalonyl-CoA (b) was generated from succinyl-CoA formed via Reactions 1 and 2. The methylmalonyl-CoA (a) formed as a result of racemase action was converted to propionyl-CoA and oxaloacetate through the transcarboxylase reaction (Reaction 4). The oxaloacetate was converted to malate with malic dehydrogenase (Reaction 5) and the extent of the reaction was determined from the decrease in absorbancy of the DPNH at 340 μm. All of the enzymes used in the assay were free of racemase except the isomerase which had a specific activity of 0.01 assayed as racemase. Consequently, isomerase could not be added in great excess. In practice, two concentrations of the racemase preparation and a control, with no addition of isomerase, were assayed simultaneously. The rate of DPNH oxidation was dependent on and proportional to the amount of racemase added (Fig. 2). Direct spectrophotometric assay was permissible in the crude extracts because at the dilutions needed for the assay the concentration of DPNH oxidase was very low.

Role of Racemase in Linking Transcarboxylase and Isomerase—The dependency of DPNH oxidation on the addition of racemase in the assay demonstrates the facts that the methylmalonyl-CoA (b) generated by the isomerase (Reaction 2) is not the substrate for the transcarboxylase and that the over-all conversion can occur only when racemase is added.

It can also be shown that the methylmalonyl-CoA (a) which is formed in the presence of transcarboxylase is not the substrate for the isomerase. This can be done spectrophotometrically by generating methylmalonyl-CoA (a) from propionyl-CoA and oxaloacetate with the aid of transcarboxylase and lactic dehydrogenase as illustrated below.

\[
\text{Propionyl-CoA} + \text{oxaloacetate} \xrightarrow{\text{transcarboxylase}} \text{methylmalonyl-CoA (a)} + \text{pyruvate}
\]

\[
\text{Pyruvate} + \text{DPNH} + \text{H}^+ \xrightarrow{\text{lactic dehydrogenase}} \text{lactate} + \text{DPN}^+
\]

If propionyl-CoA is present in a limiting amount, the formation of pyruvate and oxidation of DPNH will terminate when the propionyl-CoA is utilized completely. In the presence of racemase, isomerase, propionate, and CoA transferase, the propionyl-CoA is regenerated by the following reactions.

\[
\text{Methylmalonyl-CoA (a)} \xrightarrow{\text{racemase}} \text{methylmalonyl-CoA (b)}
\]

\[
\text{Methylmalonyl-CoA (b)} \xrightarrow{\text{isomerase}} \text{DBC, succinyl-CoA}
\]

\[
\text{Succinyl-CoA} + \text{propionate} \xrightarrow{\text{CoA transferase}} \text{propionyl-CoA} + \text{succinate}
\]

If racemase is absent the sequence will not occur because the methylmalonyl-CoA (a) is not the substrate for the isomerase and neither methylmalonyl-CoA (a) nor methylmalonyl-CoA (b) is a substrate for the CoA transferase. The over-all reaction is as follows.

\[
\text{Oxaloacetate} + \text{propionate} + \text{DPNH} + \text{H}^+ \xrightarrow{\text{lactic dehydrogenase}} \text{lactate} + \text{succinate} + \text{DPN}^+
\]

Fig. 3, Curve A shows that in the absence of racemase (0 to 4 minutes) DPNH oxidation proceeds at a very slow rate, because the isomerase does not convert the methylmalonyl-CoA (a) to succinyl-CoA. On the addition of racemase a rapid conversion occurs due to the formation of the methylmalonyl-CoA (b). The reactions proceed to completion because it is “pulled” by lactic dehydrogenase. Attempts to assay racemase by these reactions have not been successful. The conversion was not linear over a sufficiently long period of time, nor was it linear with increasing concentrations of racemase. The reason for this may be due to the small amount of transcarboxylase used.

Chemically synthesized methylmalonyl-CoA contains an equal mixture of both stereoisomers. Methylmalonyl-oxaloacetic transcarboxylase acts on only one of these forms. Fig. 3, Curve B shows the results obtained when chemically synthesized methylmalonyl-CoA was the substrate for transcarboxylase and the reaction was linked to malic dehydrogenase (Reactions 3, 4, 5). When racemase was omitted the reaction proceeded rapidly until 50% of the methylmalonyl-CoA was utilized (0 to 1 minute). Thereafter the slow rate of methylmalonyl-CoA utilization (1 to 5 minutes) may be due to spontaneous racemization of the substrate. When at 5 minutes racemase was added the reaction proceeded rapidly to completion. If racemase was added initially (Fig. 3, Curve C), the methylmalonyl-CoA was utilized completely, but the rate of the reaction decreased when the substrate concentration became limiting.

The methylmalonyl-CoA which is formed by the catalytic action of propionyl carboxylase, from animal tissue (14), is the isomer used by methylmalonyl-oxaloacetic transcarboxylase. When propionyl carboxylase is and the transcarboxylase are “linked” a rapid reaction occurs (Fig. 4, Curve A). If the methylmalonyl-CoA (a) produced with propionyl carboxylase is heated, thus converting part of the CoA ester to the inactive

3 The propionyl carboxylase preparation was a gift of Dr. Y. Kaziro and Dr. S. Ochoa of New York University School of Medicine.
The role of racemase in linking transcarboxylase and isomerase.

Curve A. Methylmalonyl-CoA (b) generated from succinyl-CoA. The reaction does not occur at a significant rate until racemase is added at 4 minutes. The reaction mixture contained in micromoles: Tris-HCl, pH 7.4, 10.0; oxaloacetate, 0.4; DPNH, 0.075; DBC, 0.001; Na propionate, 2.0; propionyl-CoA, 3.6 X 10^-2; and in units: lactic dehydrogenase, 0.1; methylmalonyl isomerase, 0.06; propionyl-CoA transferase, 0.07; methylmalonyl-oxaloacetic transcarboxylase, 3.3 X 10^-3; and methylmalonyl racemase, 7.0 X 10^-2. Final volume = 0.31 ml. The rate of this reaction was corrected for a control conducted without propionyl-CoA; this was necessary because of spontaneous breakdown of the oxaloacetate.

Curve B. Chemically synthesized methylmalonyl-CoA. Racemase was added at 5 minutes, the (b) form was converted to the (a) form, and the transcarboxylase reaction continued. The reaction mixture contained in micromoles: Tris-HCl, pH 7.4, 10.0; Na pyruvate, 2.5; reduced glutathione, 1.3; DPNH, 0.04; (NH₄)₂SO₄, 10.0; methylmalonyl-CoA, 1.0 X 10^-3; and in units: malic dehydrogenase, 0.1; methylmalonyl-oxaloacetic transcarboxylase, 6.5 X 10^-2; and methylmalonyl racemase, 7.0 X 10^-2. The final volume was 0.31 ml.

Curve C. Chemically synthesized methylmalonyl-CoA with racemase added at zero time. The reaction mixture was the same as that detailed for B.

Mechanism of the Racemization of Methylmalonyl-CoA—There are two possible mechanisms for the racemization as shown by the following equations.

Mechanism I would involve a rearrangement of hydrogen atom about carbon atom 2 of methylmalonyl-CoA. Mechanism II represents the transfer of the coenzyme A moiety between the two carboxyl groups (i.e. carbon atoms 1 and 3). During the course of this study it has been demonstrated by Overath, Kellerman, and Lynen (11) and Mazumder et al. (9) that the racemization occurs by Mechanism I. Nevertheless the results of the studies reported here showing that the racemization does not occur by Mechanism II will be reported briefly. Hegre, Miller and Lane (15) also have shown that there is little or no randomization of the coenzyme during the isomerization of methylmalonyl-CoA to succinyl-CoA and since their isomerase contained racemase the same conclusion may be drawn from their results.

Methylmalonyl-CoA labeled in the free carboxyl group with C¹⁴ (i.e. carbon 3) was prepared. The methylmalonyl-CoA-3-C¹⁴ was treated with racemase and then the CoA ester converted to the amide. The methylmalononic acid was then degraded to determine the position of the C¹⁴. If the conversion was via Mechanism I the free carboxyl should retain all the radioactivity; if by II half of the radioactivity should be in the esterified carboxyl carbon.

Methylmalonyl-CoA-3-C¹⁴ was prepared enzymatically with crystalline propionyl carboxylase. The incubation mixture contained in micromoles: propionyl-CoA, 4.5; KHC₁⁴O₃, 100; ATP, 20; MgCl₂, 20; reduced glutathione, 1.5; Tris-HCl buffer, pH 7.5, 300; and 2.6 units of propionyl carboxylase in a total volume of 3.2 ml. The reaction at 30° was complete in 10 minutes as determined spectrophotometrically with the use of transcarboxylase and malic dehydrogenase (13). The mixture was then deproteinized with 1.0 ml of 2 N perchloric acid. The excess propionyl carboxylase was removed by deproteinization, 50% of the methylmalonyl-CoA was found as isomer (b) and did not react with transcarboxylase.

Mechanism of the Racemization of Methylmalonyl-CoA—There are two possible mechanisms for the racemization as shown by the following equations.

Mechanism I would involve a rearrangement of hydrogen atom about carbon atom 2 of methylmalonyl-CoA. Mechanism II
tract containing methylmalonyl-CoA was divided into three samples: (A) 1.12 ml were held at 0°; (B) 1.12 ml were heated at 100° for 3 minutes; and (C) 2.25 ml were incubated at 30° for 15 minutes with 14 units of purified racemase. An aliquot of each sample was tested to determine the degree of racemization of the methylmalonyl-CoA. In Sample A all the methylmalonyl-CoA was present as the isomer active for transcarboxylase (Fig. 4, Curve A). Sample B was found to contain 58 to 60% methylmalonyl-CoA (a) (Fig. 4, Curve B). Evidently complete racemization as observed by Mazumder et al. (8) with similar treatment was not obtained in this experiment. In Sample C there was complete racemization of the methylmalonyl-CoA (Fig. 4, Curve C). This sample was deproteinized with perchloric acid (3 M, final concentration).

The methylmalonyl-CoA present in the remainder of each sample was then converted to the amide by treatment with ammonium hydroxide and then 1 millimole of unlabeled carrier methymalonyl-CoA was added. The methymalonyl-CoA was extracted with ether and purified by chromatography on a Celite column (16). An aliquot of the purified methymalonyl-CoA was oxidized to CO₂ (17) and the radioactivity determined on a gas proportional counter. The remainder of the sample was degraded by the Hoffman method (18). The CO₂ was collected and the α-alanine in the residue was degraded with ninhydrin to acetaldehyde and CO₂. Typical results are shown in Table II. Considerable C⁴ appeared in the CO₂ from the Hoffman degradation. In five experiments an average of 25% of the total radioactivity of the original methymalonyl-CoA was found in the CO₂ from the amide but there was no difference in the percentage of C⁴ found in the CO₂ from the racemase-treated and the control samples. In most cases the α-carboxyl of the alanine had a specific activity equal to that of the original methymalonyl-CoA. This result indicated that the C⁴ found in the CO₂ in the Hoffman degradation was due to a contamination from the decarboxylation of the methymalonyl-CoA. The nonspecificity was rather constant (approximately 25%). Since there was no difference between racemized and unracemized samples, it is concluded that no CoA transfer either of an inter- or an intramolecular nature occurred during racemization.

**Heat Stability of Racemase**—Methylmalonyl racemase is unusually resistant to heating. Tubes containing 26 μg of racemase (specific activity = 8.0) and 60 μmoles of Tris-HCl buffer, pH 7.4, in a total volume of 0.06 ml were placed in a water bath at 100° and removed after 1, 5, 10, and 20 minutes. After appropriate dilution the samples were tested for racemase activity. Compared to that of an unheated control, 67, 59, 9, and 0% of the activity remained after boiling for 1, 5, 10, and 20 minutes, respectively. These results indicate that 50% inactivation required approximately 5 minutes and complete inactivation 10 to 20 minutes in the boiling water bath. A white precipitate formed in the tubes during heating. In a second experiment, the racemase sample which had been heated 10 minutes was chilled and this precipitate sedimented by centrifugation. The fact that the clear supernatant solution was found to contain the racemase activity showed that the enzyme remained soluble.

**Stability of Racemase to Perchloric Acid**—Methylmalonyl-CoA (a) prepared with crystalline propionyl carboxylase as described above was treated with racemase and the resulting mixture containing both (a) and (b) forms of methylmalonyl-CoA was adjusted to 1.0 M with perchloric acid. After 15 minutes at 0° the mixture was neutralized with 2 N KOH and the precipitate was removed by centrifugation. The solution was then assayed for methymalonyl-CoA with transcarboxylase and malic dehydrogenase, both free of racemase. All the methymalonyl-CoA was utilized by the transcarboxylase because some of the racemase was still active. When 3.0 M perchloric acid (final concentration) was used equal quantities of methymalonyl-CoA (a) and (b) (Fig. 4, Curve C), were demonstrated. Racemase is resistant to 1.0 M perchloric acid for as long as 30 minutes at 0° but is destroyed by 3.0 M perchloric acid in 20 minutes at 0°.

**Equilibrium of Racemase Reaction**—Methylmalonyl-CoA racemase (14 units, specific activity, 31) was incubated at 30° for 15 minutes with 3.0 μmoles of methylmalonyl-CoA (a) which was prepared with propionyl-CoA carboxylase. Equilibrium should have been reached in less than 1 minute. Assay of an aliquot (Fig. 4, Curve C) of the equilibrium mixture after deproteinization with 3 M perchloric acid showed an equal mixture of the two isomers. Calculations showed 1.35 μmoles of methylmalonyl-CoA (a) and 1.35 μmoles of (b). The equilibrium concentrations of the two substances is therefore

\[
\text{Methylmalonyl-CoA (a)} = 1.0
\]

\[
\text{Methylmalonyl-CoA (b)} = 1.0
\]

Synthetic (i.e. chemically prepared) methylmalonyl-CoA also contains a 50:50 mixture of the two isomers (Fig. 3, Curve B). Racemization by heating gives an equal mixture of the two isomers as reported by Mazumder et al. (8) and Overath, Kellermeyer, and Lynen (11).

**Other Properties of Racemase**—No cofactor requirements for the pure enzyme have been found. The enzyme is stable to dialysis, and has been stored in 0.05 M Tris-HCl buffer, pH 7.4, for 9 months at -10° without loss of activity.
Methylmalonyl racemase is difficult to separate from the other enzymes which have been isolated from Propionibacterium shermanii. The methylmalonyl-oxaloacetic transcarboxylase, propionyl-CoA transferase, and methylmalonyl isomerase all contained racemase until they were extensively purified. The dependence on racemase for the coupling of isomerase and transcarboxylase could be demonstrated only with highly purified enzymes.

Racemase on the other hand, because of its heat stability, is quite easily freed of other enzymes. In the purification procedure the recovery of racemase from the cellulose phosphate column was quite low (approximately 13%). In the purification of transcarboxylase (13) a large loss (50%) also occurred at this step. The use of cellulose phosphate is important, since it adsorbs the racemase and transcarboxylase while allowing most of the other enzymes to pass through the column. Perhaps some of the transcarboxylase and racemase are bound virtually irreversibly to the cellulose phosphate and thus lost in the purification at this step.

The teleological role of racemase in propionibacteria and more especially in the production of propionate by these bacteria is of interest and may be considered in relation to the cyclic scheme of propionate formation shown in Fig. 5. A rapid formation of propionate should depend upon the saturation of transcarboxylase with its substrate in correct isomeric form, methylmalonyl-CoA (a). Since the $K_m$ for methylmalonyl CoA (a) with transcarboxylase is about $1 \times 10^{-5} \text{M}$ (13) this saturation would appear to be easily accomplished. However, the concentration of methylmalonyl-CoA (b) formed from succinyl-CoA may be low because the equilibrium for the isomerase is approximately 20:1 in favor of succinyl-CoA, and the $K_m$ for succinyl-CoA with the isomerase is $3.3 \times 10^{-3} \text{M}$ as compared with the $K_m$ for methylmalonyl-CoA (b) of $8 \times 10^{-3} \text{M}$. Thus the production of a small amount of the methylmalonyl-CoA (b), the wrong isomer for transcarboxylase, could limit the rate of the transcarboxylation step. In such a case rapid racemization of methylmalonyl-CoA would be important.

Although no evidence is available at this time, methylmalonyl-oxaloacetic transcarboxylase which is active with either acetyl-CoA or propionyl-CoA as carboxyl acceptors may be an important enzyme in the synthesis of fatty acids in propionibacteria and perhaps in other tissue. Methylmalonyl-CoA has been suggested as a precursor in the formation of branched chain fatty acids (19-21). Indication that methylmalonate can be incorporated into the branched chain lactone of erythromycin has been presented (22). If the enzymes condensing methylmalonyl-CoA into fatty acid are stereospecific for one isomer, either the (a) or the (b) form, then methylmalonyl racemase might be an important enzyme for the synthesis of branched chain fatty acids.

**SUMMARY**

Methylmalonyl racemase of Propionibacterium shermanii has been isolated and purified to the point of homogeneity as judged by sedimentation and electrophoresis. It has a sedimentation constant $s_{20,w} = 9.9 \text{S}$; the electrophoretic mobility is $8.4 \times 10^{-4} \text{cm}^2 \text{per second per volt at pH 7.4}$. The enzyme apparently has a low molecular weight. A spectrophotometric assay is described for this enzyme. In agreement with other observations (8-11), the enzyme seems to catalyze the interconversion of the two isomers of methylmalonyl-CoA (designated as (a) and (b)). Methylmalonyl-oxaloacetic transcarboxylase is specific for methylmalonyl-CoA (a) whereas methylmalonyl isomerase is specific for isomer (b), and racemase serves to couple these two enzymes. The racemase does not function by transfer of the CoA moiety between carboxyl groups of methylmalonyl-CoA. The enzyme is unusually stable to heat and to perchloric acid treatment. The concentrations of the stereo-isomers, methylmalonyl-CoA (a) and (b), are equal at equilibrium conditions in the presence of racemase.

**Acknowledgment**—The authors thank Miss Doris A. Roberts for performing the electrophoresis and ultracentrifuge studies.

**REFERENCES**

The Isolation, Purification, and Properties of Methylmalonyl Racemase
S. H. G. Allen, R. Kellermeyer, Rune Stjernholm, Birgit Jacobson and Harland G. Wood


Access the most updated version of this article at http://www.jbc.org/content/238/5/1637.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/238/5/1637.citation.full.html#ref-list-1