Malonyl Coenzyme A Synthetase
PURIFICATION AND PROPERTIES

Yu Sam Kim and Son Kwon Bang
From the Department of Biochemistry, College of Science, Yonsei University, Seoul, Korea

Malonyl coenzyme A synthetase (EC 6.2.1.14) was induced in Pseudomonas fluorescens grown on malonate as a sole carbon source. This enzyme was purified, for the first time, over 30-fold by the combination of ammonium sulfate precipitation, Sephadex G-150 gel filtration, DEAE-Sephacl ion exchange chromatography, and hydroxylapatite chromatography. The purified enzyme, which had a specific activity of about 0.512 μmol/min/mg, appeared to be electrophoretically homogeneous. The molecular size of the enzyme was determined to be 98,000 Da which is composed of two 49,000-Da subunits. The optimum pH for the enzyme was 7.5. Malonyl coenzyme A synthetase requires ATP, CoA, and Mg2+ for the full enzyme activity. With succinate or acetate, the synthetic rate of CoA derivative was 40% of that observed with malonate. The malonyl coenzyme A synthetase showed typical Michaelis-Menten kinetics for the substrate, malonate, and malonyl-CoA. The K_m values were calculated to be 3.8 × 10^{-4} M, 2 × 10^{-3} M, and 10^{-3} M and V_{max} values to be 0.117 μmol/min/mg, 0.111 μmol/min/mg, and 0.142 μmol/min/mg, respectively. The purified malonyl coenzyme A synthetase was immunogenic in the rabbit and Ouchterlony double diffusion analysis revealed a single precipitant line with the antiserum. The antisera inhibited the enzyme activity and the extent of inhibition was dependent on the amount of the serum added.

The existence of a system synthesizing malonyl-CoA directly from malonate has long been indicated in the biological system. Lara (1) and Hayashi and Kornberg (2) showed that malonate was formed as a degradation product of pyrimidines by bacteria, indicating this compound exists in the biological system and could arise in normal catabolism. Gray (3) has shown that dry cells of a malonate-adapted strain of Pseudomonas aeruginosa are capable of degrading malonate anaerobically to CO2 and acetate. However, Hayashi (4) showed the involvement of CoA and ATP in the decarboxylation of malonate and suggested that either acetyl-CoA or CoA or acetate and an active 1-carbon fragment could be the primary decarboxylation product. Wolfe et al. (5) and Hayashi (6) had reported independently on the enzymatic degradation of malonate from malonate-grown Pseudomonas fluorescens. In addition to bacteria, it has been reported that malonate was activated in rat tissues such as liver, kidney, and heart in the presence of ATP and CoA to form malonyl-CoA, which is subsequently degraded to acetyl-CoA and CO2 (7). Such a result was also obtained from the study of malonate metabolism in rat brain mitochondria (8) and from plants (9). Recently, Kim and Kolattukudy (10) reported that malonyl-CoA decarboxylase is induced in P. fluorescens grown on malonate as a sole carbon source, strongly indicating the existence of an enzyme system synthesizing malonyl-CoA. In this bacteria, the key enzymes in the glyoxylate cycle, isocitrate lyase (11) and malate synthase, were also known to be induced. In malonate metabolism, one of the most important steps is the activation of malonate to malonyl-CoA. However, little is known about this enzyme system except that Koeppe et al. (8) have indirectly characterized it in the rat brain mitochondrial extract and malonyl-CoA synthetase (1, 6, 14) has never been purified in any biological system. In this paper, we describe, for the first time, purification and characterization of malonyl-CoA synthetase from P. fluorescens grown on malonate as a sole carbon source.

EXPERIMENTAL PROCEDURES

Materials
Sodium malonate, ATP, CoA, ADP, AMP, GTP, DEAE-Sephalac, and bovine serum albumin were purchased from Sigma. Hydroxyapatite and Coomassie Brilliant Blue R-250 were obtained from Bio-Rad Laboratories. Sephadex G-150 was purchased from Pharmacia Fine Chemicals. Acrylamide, bisacrylamide, Temed, and ammonium persulfate were purchased from Bethesda Research Laboratories. All other reagents were obtained commercially and were of analytical grade.

Methods
Growth of Bacteria—P. fluorescens ATCC 11250 was grown on a medium containing malonate. The medium has the following composition: 0.3% NH4Cl, 1% sodium malonate, 0.2% KH2PO4, 0.04% MgSO4·6H2O, 0.001% FeSO4·7H2O. P. fluorescens, a strictly aerobic microorganism, was grown approximately 24 h at 30 °C with constant mechanical shaking. The cells were harvested in late log phase, when the density of the culture was equivalent to about 5 g (wet weight) of cells per liter. The cell paste was collected in a refrigerated preparative centrifuge and stored in a freezer until used.

Enzyme Assay—Malonyl-CoA synthetase was assayed by measuring the rate of production of malonyl-CoA through the formation of malonohydroxamate (12). The reaction mixtures containing (in micromoles) potassium phosphate buffer, pH 7.2, 100; MgCl2, 20; NH4OH (neutralized with KOH), 200; ATP, 10; CoA, 0.2; and enzyme (final volume, 1 ml) were incubated at 30 °C for 30 min. The reaction was terminated by the addition of 0.5 ml of 10% trichloroacetic acid. Then, 1 ml of 15% FeCl3·6H2O in 0.66 N HCl was added to the reaction mixture to develop the red-brown color representing the malonohydroxamate-Fe3+ complex. The protein precipitate was removed by centrifugation and the color was measured at 540 nm by a spectronic 20 spectrophotometer. The molar extinction coefficient

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2 The abbreviations used are: Temed, N,N,N',N'-tetramethylthlyl-
ylenediamine; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography.
obtained from the standard curve plotted Amax versus malonylhydroxamate from malonyl-CoA was 8.42 × 10⁻⁴.

One unit of malonyl-CoA synthetase is defined as the amount of enzyme that catalyzes the formation of 1 µmol of malonylhydroxamate per min in this assay. Protein was determined by the method of Lowry et al. (12).

Preparation of Cell-free Extract and Ammonium Sulfate Precipitation—*P. fluorescens* cells (wet weight 50 g), kept in a refrigerator, were thawed and resuspended in 5 volumes of 50 mM potassium phosphate, pH 7.2, containing 1 mM 2-mercaptoethanol and acid-washed glass beads (Sigma, diameter 75–150 µm). The cell suspension was then chilled to 0 °C, subjected to ultrasonic treatment (Lab-Line Instruments, Inc.) for 3-min intervals for a total of 10 min, and centrifuged at 20,000 × g for 15 min. The pellet was resuspended in the same buffer, the extraction was repeated three times, and the supernatants were pooled. To the pooled supernatant, 80% saturated ammonium sulfate solution was slowly added with stirring until 30% saturation was achieved. The precipitated protein was collected by centrifugation, dissolved in 10–15 ml of 0.1 M potassium phosphate buffer, pH 7.2, containing 1 mM 2-mercaptoethanol, and dialyzed for 6 h against the same buffer, and any insoluble material was removed by centrifugation.

**Gel Filtration on Sephadex G-150—**The enzyme solution obtained from the first step was subjected to gel filtration on a Sephadex G-150 column (2.8 × 15 cm) pre-equilibrated with 0.1 M potassium phosphate buffer, pH 7.2, containing 1 mM 2-mercaptoethanol, and proteins were eluted in a descending manner with the same buffer collecting 4-ml fractions/15 min. The fractions containing malonyl-CoA synthetase were pooled, concentrated using Millipore XM-10 membrane filters, dialyzed overnight against 20 mM Tris-HCl buffer, pH 7.6, containing 1 mM 2-mercaptoethanol, and any insoluble material was removed by centrifugation at 20,000 × g for 15 min.

**Anion Exchange Chromatography on DEAE-Sephacel—**The enzyme solution, obtained from the previous step, was applied directly to a DEAE-Sephacel column (2.8 × 15 cm), which had been equilibrated with 20 mM Tris-HCl buffer. Following sample application, the column was washed by using 10 void volumes of 20 mM Tris-HCl buffer. After washing the column, the bound proteins were eluted with a step gradient of 0 to 0.1 M, 0.15 M, 0.2 M, 0.25 M, and 0.3 M potassium phosphate buffer (pH 7.6) in a total volume of 90 ml of Tris-HCl buffer, pH 7.6. Each fraction had little malonyl-CoA synthetase activity, but the mixture, the fraction eluted with 0.15 M KCl, and that with 0.3 M KCl, showed original enzyme activity. The enzyme fraction was concentrated and dialyzed overnight against 5 mM potassium phosphate buffer, pH 7.2, containing 1 mM 2-mercaptoethanol, and any insoluble material was removed by centrifugation.

**Hydroxyapatite Column Chromatography—**The enzyme solution obtained from the previous step was applied to a hydroxyapatite (Bio-Rad) column (2.8 × 10 cm) equilibrated previously with 5 mM potassium phosphate buffer, pH 7.2, containing 1 mM 2-mercaptoethanol. After washing the column with the same buffer (10 void volumes), the absorbed proteins were washed with potassium phosphate buffer, pH 7.2, using a 150-ml linear gradient (5–30 mM) at a flow rate of about 3 ml/12 min. The fractions containing the majority of malonyl-CoA synthetase were pooled, stored at 0 °C, and used for all subsequent experiments.

**Polyacrylamide Gel Electrophoresis—**Polyacrylamide disc gel electrophoresis was performed with 7% acrylamide according to the procedure of Laemmli (14). The gel system consisted of 7% acrylamide resolving gel (8 cm, pH 8.8) and a 3% stacking gel (1.5 cm, pH 6.8). About 10 to 50 µg of protein was applied to the gel and the slab gel (8 × 12 × 0.1 cm) electrophoresis was performed at 50 to 100 V for 4 h.

**SDS-Polyacrylamide Gel Electrophoresis—**The purified malonyl-CoA synthetase was precipitated by adding trichloracetic acid solution (final concentration, 10%), washed three times with acetone, and collected. Samples were denatured at 0.0625 M Tris-HCl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.01% bromphenol blue by heating at 100 °C for 3 min prior to loading on gel. Electrophoresis was performed by the Laemmli method. The polyacrylamide running gel was 10% with sucrose. Molecular size markers (in daltons) were bovine serum albumin (66,000), ovalbumin (45,000), pepsin (34,700), β-lactoglobulin (18,400), and lysozyme (14,300).

**Molecular Size Determination—**The molecular size of malonyl-CoA synthetase was determined by gel filtration with HPLC protein column 1-250, Waters Inc. The column was equilibrated with 50 mM sodium phosphate (pH 6.8) buffer and calibrated with blue dextran 2000 and standard proteins such as γ-globulin (150,000), human serum albumin (66,000), ovalbumin (45,000), and myoglobin (17,200).

**Identification—**Malonylhydroxamate was identified as an enzyme reaction product by ascending paper chromatography with water-saturated butanol as described before (15).

**Preparation of Antiserum—**Antiserum was prepared by immunizing rabbits with purified malonyl-CoA synthetase. About 170 µg of purified enzyme in 1 ml of 0.1 M potassium phosphate buffer was emulsified by sonication with 1 ml of complete Freund's adjuvant and the mixture was subcutaneously injected into a rabbit. One week after the first injection, 100 µg of the enzyme emulsified with 1 ml of incomplete Freund's adjuvant was injected into the same rabbit. Two weeks after the second injection, the rabbit was bled from the ear and the antiserum was collected.

**Immunodiffusion—**The specificity of the antibody was determined by the Ouchterlony immunodiffusion technique (16) using 1% agar prepared in 0.85% NaCl. Diffusion was terminated in 10 h and nonagglutinated protein was removed from the agar plate by repeated washing with 0.85% NaCl. Then, the immunoprecipitant band was fixed with 7.5% acetic acid and stained with Amido Black.

**RESULTS**

**Growth of *P. fluorescens* and Induction of Malonyl-CoA Synthetase**

*P. fluorescens* were grown on the medium containing malonate as a sole carbon source. The rate of cell growth was considerably increased by the addition of Fe²⁺ ion. During the period of cell growth on malonate, malonyl-CoA synthetase was induced, whereas on glucose it was not. The activity of the enzyme was the highest at the late logarithmic phase.

**Purification of Malonyl-CoA Synthetase**

The crude extract was prepared by the sonication of the cells harvested at late log phase. The specific activity of this crude extract was about 15.8 mmol/min/mg. Bulk of the malonyl-CoA synthetase in the crude extract was recovered in the protein precipitated between 30 and 50% saturation with ammonium sulfate and this step resulted in nearly a 2.5-fold purification with 70% recovery of the total activity. This enzyme preparation was applied on a Sephadex G-150 filtration. Gel filtration of this protein revealed two major protein peaks in which the protein eluted in the first peak contained most of the enzyme activity (Fig. 1). This step resulted in 86% recovery of the enzyme with 7.3-fold purification. When the enzyme solution obtained from the above step was applied to the DEAE-Sephacel column, most of the malonyl-CoA synthetase was absorbed. The proteins were eluted as two major protein peaks by increasing the concentration of KCl to 0.3 M. However, most of the enzyme activity disappeared except for a very low enzyme activity peak at the shoulder of the second protein peak (Fig. 2). Most of the malonyl-CoA synthetase was recovered when the active fraction was mixed with the fractions eluted in the leading shoulder of the first protein peak indicating the dissociation of subunits (Table I). Even though the recovery of the enzyme activity was low, this step resulted in a 1.5-fold purification. When the enzyme solution was applied on a hydroxyapatite column, nearly 80% of the protein was bound. The bound protein was eluted by increasing the concentration of phosphate, 5 mM to 300 mM. One major protein peak appeared by this elution and the enzyme activity coincided exactly with the protein peak eluted at about 120 mM phosphate concentration (Fig. 3). This step resulted in about a 3.5-fold purification with 55% recovery of the enzyme. Thus, the combination of the five steps resulted in about a 32-fold purification of...
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FIG. 1. Sephadex G-150 gel filtration chromatography of malonyl-CoA synthetase. Malonyl-CoA synthetase from the ammonium sulfate fraction (30 to 50%) step was loaded onto a gel column (3 x 90 cm). Four ml fractions were collected throughout and the protein peak was monitored by absorbance at 280 nm (O--O). Malonyl-CoA synthetase activity (●-●) was assayed as described under "Methods."

FIG. 2. Anion exchange chromatography of malonyl-CoA synthetase on DEAE-Sephacel. Malonyl-CoA synthetase from the gel filtration step was applied to a DEAE-Sephacel anion exchanger column (2.8 x 15 cm). The column was washed with 20 mM Tris-HCl, pH 7.6, containing 2-mercaptoethanol, to remove weakly bound protein. Absorbance at 280 nm (O--O) and malonyl-CoA synthetase activity (●-●) were measured. The dotted line (-----) represents salt (KCl, gradient concentration. A, B, C, D, and E show the fractions for the regeneration experiment of malonyl-CoA synthetase activity (refer to Table I).

TABLE I

Regeneration of malonyl-CoA synthetase activity

Malonyl-CoA synthetase activity was recovered by mixing the fractions, which are eluted by the linear gradient from DEAE-Sephacel ion exchange column. A, B, C, D, and E represent the fractions eluted and pooled from the DEAE-Sephacel column shown on Fig. 2.

<table>
<thead>
<tr>
<th>Fraction mixed</th>
<th>Malonyl-CoA synthetase activity (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E + A</td>
<td>0.063</td>
</tr>
<tr>
<td>E + B</td>
<td>0.173</td>
</tr>
<tr>
<td>E + C</td>
<td>0.026</td>
</tr>
<tr>
<td>E + D</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Malonyl-CoA synthetase with overall recovery of nearly 5% (Table II). The rate of malonyl-CoA synthesis by the purified enzyme was linear up to at least 30 min and 300 µg of protein per ml. In all experiments, assays were done within the linear range.

Criteria of Purity of Enzyme

The purity of the malonyl-CoA synthetase preparation resulting from the final step of hydroxylapatite chromatography was acceptable. The enzyme was free of contaminating proteins as judged by polyacrylamide disc gel electrophoresis and staining with Coomassie Blue.

FIG. 3. Hydroxylapatite chromatography of malonyl-CoA synthetase. DEAE-Sephacel-purified malonyl-CoA synthetase was loaded on a hydroxylapatite column (2.8 x 10 cm) equilibrated with 5 mM potassium phosphate buffer, pH 7.2, containing 1 mM 2-mercaptoethanol. Activity was eluted with a linear gradient ranging from 5 mM to 300 mM potassium phosphate, pH 7.2. Protein peak (O--O) and malonyl-CoA synthetase (●-●) were monitored. The dotted line (-----) represents phosphate gradient concentration.

TABLE II

Purification of malonyl-CoA synthetase from malonate grown on P. fluorescens

<table>
<thead>
<tr>
<th>Stage of purification</th>
<th>Protein (mg)</th>
<th>Total activity (units x 10^6)</th>
<th>Specific activity (units/mg)</th>
<th>Recovery (%)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>2.464</td>
<td>38,900</td>
<td>15.8</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ (30-50%)</td>
<td>660</td>
<td>26,400</td>
<td>40.1</td>
<td>70</td>
<td>2.50</td>
</tr>
<tr>
<td>Sephadex G-150</td>
<td>197.8</td>
<td>22,700</td>
<td>115.2</td>
<td>55.5</td>
<td>7.30</td>
</tr>
<tr>
<td>DEAE-Sephacel</td>
<td>19.7</td>
<td>3,410</td>
<td>172.9</td>
<td>8.7</td>
<td>11.00</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
<td>3.6</td>
<td>1,876</td>
<td>512.0</td>
<td>4.8</td>
<td>32.40</td>
</tr>
</tbody>
</table>

FIG. 4. Polyacrylamide disc gel electrophoresis showing the progress of the purification of malonyl-CoA synthetase. Acrylamide gels (7%) were run as described under "Experimental Procedures" and stained with Coomassie Blue. Gel A shows the 20,000 x g supernatant, B shows the ammonium sulfate fraction, C shows the pooled fraction of the Sephadex G-150 column, D represents DEAE-Sephacel-eluted enzyme, and E represents the purified enzyme from the final hydroxylapatite step.
**FIG. 5.** SDS-polyacrylamide gel electrophoresis of the purified malonyl-CoA synthetase-obtained hydroxylapatite step. Enzyme sample(s) were denatured in 2% SDS, 5% 2-mercaptoethanol for 3 min at 100 °C, and loaded onto a 10% acrylamide gel. The procedure used the Laemmli method. The S lane has one single band suggesting that the enzyme fraction was homogeneous. The M lane shows marker proteins. A, bovine serum albumin (68,000); B, ovalbumin (45,000); C, pepsin (34,700); D, β-lactoglobulin (18,400); E, lysozyme (14,300).

**FIG. 6.** Ouchterlony immunodiffusion analysis of the purified malonyl-CoA synthetase. The right well (B) contained rabbit antiserum prepared against homogeneous malonyl-CoA synthetase. The left well (A) contained malonyl-CoA synthetase (20 μg) from crude extract.

**FIG. 7.** Product identification by UV spectrophotometer. The UV spectral change was monitored between A (reaction mixture except malonate) and B (complete reaction mixture). A and B were scanned from 300 nm to 190 nm. After scanning, the B line had an absorbance at the 235 nm because of thioester bond formation.

**Table III**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Activity (nmol/min)</th>
<th>Relative rate (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>Complete system −CoA</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>Complete system −ATP</td>
<td>1.5</td>
<td>9.3</td>
</tr>
<tr>
<td>Complete system −MgCl₂</td>
<td>8.9</td>
<td>55</td>
</tr>
</tbody>
</table>

**Cofactor Requirement**

For maximum enzyme activity, malonyl-CoA synthetase needs cofactors. The complete system contained CoA (0.2 μmol), ATP (10 μmol), malonate (40 μmol), MgCl₂ (20 μmol), 2-mercaptoethanol (1 μmol), Tris-HCl (pH 7.6, 100 μmol) buffer, enzyme (100 μl), and water to make a total volume of 1.0 ml. Enzyme assay was measured as described under "Methods."

**Product Identification**

Since the enzyme catalyzes the formation of malonyl-CoA from malonate and coenzyme A, the formation of thioester bond was measured directly by following the increase in absorbance at 235 nm or by converting the thioester to a hydroxamate. As shown on Fig. 7, the UV spectral change near 235 nm was monitored between the reaction mixture without malonate and the complete reaction mixture after incubation, suggesting the formation of a thioester bond. The formation of thioester bond was also confirmed by the identification of malonohydroxamate (Rₛ, 0.19) using paper chromatography.

**Cofactor Requirement**

The rate of malonate activation to malonyl-CoA by the enzyme was the highest in the presence of ATP, CoA, and Mg²⁺ ion. Practically no enzyme activity was observed when either ATP or CoA was omitted from the reaction mixture (Table III). From these observations it was elucidated that malonyl-CoA synthetase requires ATP, CoA, and Mg²⁺ ion for its full enzyme activity.

**Properties of Malonyl-CoA Synthetase**

**Molecular Size**—Molecular size of purified malonyl-CoA synthetase estimated by gel filtration using a calibrated...
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**Fig. 8.** Molecular weight determination of the purified malonyl-CoA synthetase by HPLC gel filtration chromatography. The purified malonyl-CoA synthetase was applied to a HPLC protein 1-250 column which had been precalibrated with marker proteins. To calculate the partition coefficient of a given protein, determination of the internal volume \( V_i \) and the void volume \( V_v \) are needed. The formula is as follows: \( V_e = V_i + \alpha V_v \), where \( V_e \) is the elution volume and \( \alpha \) represents the partition coefficient of a solute between the liquid phase and the stationary phase. The molecular weight markers were: A, \( \gamma \)-globulin (150,000); B, human serum albumin (68,500); C, ovalbumin (45,000); D, myoglobin (17,200). The known molecular weights are plotted against the partition coefficient.

**Fig. 9.** Determination of subunit molecular weight of malonyl-CoA synthetase. 10% acrylamide gels were used. The subunit of malonyl-CoA synthetase was determined by molecular weight markers. A, bovine serum albumin (68,000); B, ovalbumin (45,000); C, pepsin (34,700); D, \( \beta \)-lactoglobulin (18,400); and E, lysozyme (14,300).

HPLC protein I-250 column, Waters Inc., was 98,000 Da (Fig. 8). Molecular size of the subunit of the enzyme estimated by SDS-polyacrylamide gel electrophoresis was 49,000 Da (Fig. 9). Thus, malonyl-CoA synthetase appears to be a dimeric enzyme composed of two identical, in size, subunits, but amino acid composition of the subunits is different as indicated by protein separation on DEAE-Sephacel and the regeneration of the enzyme activity by the mixing of two protein fractions.

**pH Dependence**—The effect of pH on malonyl-CoA synthetase was studied using three buffer systems over a pH range of 4.0 to 9.0. As the pH increased from 5 to 7.5, the enzyme rate increased gradually and the pH optimum for the purified malonyl-CoA synthetase was 7.5 in potassium phosphate buffer. At the same pH, the potassium phosphate buffer was better than the Tris-HCl buffer (Fig. 10). The optimum pH is close to that observed with the crude preparation of enzyme from rat brain (8).

**Kinetic Constants**—With increasing the concentration of malonate, CoA, or ATP, the rate of malonyl-CoA synthesis increased and a typical Michaelis-Menten type substrate saturation pattern was obtained. The double reciprocal plots were linear. From such plots, \( k, \) and \( V_{max} \) values for malonate were 0.384 mM and 0.117 pmol/min/mg (Fig. 11), for ATP were 2 mM and 0.111 pmol/min/mg (Fig. 12), and for CoA were 0.1 mM and 0.142 pmol/min/mg (Fig. 13), respectively. This \( k, \) value for malonate and ATP is higher than those reported for a crude enzyme preparation from rat brain and the \( k, \) for CoA is considerably lower (8).

**Substrate Specificity**—The substrate specificity of the purified enzyme was determined with mono- or dicarboxylic acids (Table IV). With succinate or acetate, the synthetic rate of the CoA derivative was about 40% of that observed with malonate. This finding is accordant with the report of Hay-
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**FIG. 12.** Effects of ATP concentration on the rate of malonyl-CoA formation by purified enzymes from malonate-grown cells. A typical Michaelis-Menten substrate saturation pattern was obtained.

**FIG. 13.** Effects of CoA concentration on the rate of malonyl-CoA formation by malonyl-CoA synthetase. The double reciprocal plots were linear and from such plots \( K_m \) and \( V_{max} \) were calculated.

**TABLE IV**

Substrate specificity of malonyl-CoA synthetase

Malonyl-CoA synthetase substrate specificity on the mono- and dicarboxylic acids was determined. This enzyme activated malonate and had a 40% synthetase activity on the succinate and acetate.

<table>
<thead>
<tr>
<th>Substrate analog</th>
<th>Activity (nmol/min)</th>
<th>Relative rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malonate</td>
<td>42.5</td>
<td>100</td>
</tr>
<tr>
<td>Succinate</td>
<td>19.8</td>
<td>46.5</td>
</tr>
<tr>
<td>Acetate</td>
<td>17.8</td>
<td>42</td>
</tr>
<tr>
<td>Fumarate</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

aishi (6). No catalytic activity was detected with other dicarboxylic acids, such as glutarate, \( \alpha \)-ketoglutarate, lactate, malate, methylmalonate, oxalate, oxalacetate, and tartrate. The nucleotide specificity of the enzyme was also determined (Table V). Malonyl-CoA synthetase from *P. fluorescens* appears to be specific for ATP. GTP as a substitute of ATP was effective only to about 10%. ADP was more effective than GTP, whereas AMP was a very poor substrate.

**TABLE V**

Nucleotide specificity of malonyl-CoA synthetase

Substrate specificity of malonyl-CoA synthetase on the nucleoside di- and triphosphate was determined.

<table>
<thead>
<tr>
<th>Substrate analog</th>
<th>Concentration (mM)</th>
<th>Activity (nmol/min)</th>
<th>Relative rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>10</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>ADP</td>
<td>10</td>
<td>7.3</td>
<td>16.3</td>
</tr>
<tr>
<td>AMP</td>
<td>10</td>
<td>0.33</td>
<td>0.07</td>
</tr>
<tr>
<td>GTP</td>
<td>10</td>
<td>4.66</td>
<td>10.3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Malonyl-CoA synthetase, which catalyzes the formation of malonyl-CoA directly from malonate and coenzyme A, was isolated and characterized for the first time from malonate grown on *P. fluorescens*. The function of this enzyme in this organism is obviously the supply of carbon skeleton and energy. However, this enzyme may play another important role in a variety of tissues in consideration of the wide occurrence of malonate in the biological system. Vennesland *et al.* (18) reported that malonate is produced from oxalacetate in the presence of Mn\(^{2+}\) and a crystalline metmyoglobin preparation from horse liver. Free malonate accumulates in many plants (19), while plant tissue and extracts convert labeled malonate to CO\(_2\) and trichloroacetic acid cycle acids (20). Malonate was also shown to be a product of uracil degradation by bacterial enzymes (1, 2). Although the utilization of malonate by microorganisms (21) as well as by mammals (22) and fishes has been reported, its precise metabolism has not been elucidated. Recently, Mitzen *et al.* (23) reported that malonate-related enzymes of rat brain change developmentally and suggested that they may have a greater role in brain metabolism than currently realized. There is some evidence that the dissimilation of malonate involves its conversion to acetyl-CoA and CO\(_2\). However, this enzyme, which catalyzes the formation of CoA derivative, malonyl-CoA, has never been purified homogeneously from any
sources, even though it has been suspected as an important enzyme in some tissues. The molecular characteristic of the purified enzyme was somewhat similar to that of succinyl-CoA synthetase. There is also some evidence that this enzyme has decarboxylase activity. But, it is not clear yet whether there are two distinct active sites or there is one active site playing both roles.

REFERENCES
