Purification and properties of malonyl-CoA synthetase from *Rhizobium japonicum*

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A novel malonyl-CoA synthetase was found in *Rhizobium japonicum* bacteroid of the soybean nodule. The levels of the enzyme in the free-living cells grown on a variety of carbon sources including glucose were similar, indicating that this enzyme is not inducible. The malonyl-CoA synthetase from glucose-grown *Rhizobium japonicum* was purified to homogeneity. The M_r of the enzyme was determined to be 58 000 by gel filtration on a Sephacryl S-300 and by SDS/PAGE respectively, indicating a single polypeptide enzyme. N-Terminal amino acid of the enzyme was methionine but the enzyme preparation contained about 40% de-methionylated protein. The enzyme catalyses the formation of malonyl-CoA, AMP and PP_i directly from malonate, CoA and ATP in the presence of Mg²⁺. High substrate specificity on malonate and ATP was revealed, but Mn²⁺ could be substituted for Mg²⁺ without any difference in activity. Optimum pH was 7.9. Kinetic constants, K_m and V_{max} , for malonate, CoA and ATP were 200 μ M and 21.3 μ mol/min per mg, 87 μ M and 41.7 μ mol/min per mg, and 33.3 μ M and 29.4 μ mol/min per mg respectively. Succinate inhibited the enzyme non-competitively, whereas AMP and ADP inhibited competitively. Diethylpyrocarbonate and pyridoxal-5'-phosphate severely inhibited the enzyme, but iodoacetamide, *p*-chloromercuriphenylsulphonate, *N*-acetylimidazole and phenylglyoxal did not.

INTRODUCTION

Malonyl-CoA synthetase catalyses the formation of malonyl-CoA directly from malonate and CoA in the presence of ATP and Mg²⁺. The enzyme was first purified from *Pseudomonas fluorescens* grown on malonate as a sole source of carbon [1]. In the first stage of the catalysis, the *Pseudomonas* enzyme is phosphorylated by ATP with the release of ADP [2]. The existence of such an enzyme, especially in malonate-grown *Pseudomonas* [3] and in rat brain mitochondria [4] has long been known, as malonate occurs widely in micro-organisms, animals and plants [5,6]. Malonate may play a regulatory role in the utilization of glutamate in rat brain mitochondria [7]. The metabolism of malonate has been investigated mostly in plants, especially in the Leguminosae. In nodules from 33-day-old soybean plants, malonate is the predominant acid, and the level of the acid is depressed in response to the addition of either nitrate or ammonia [6]. The result suggests that malonate or its metabolite may play a role in symbiotic nitrogen metabolism and that there may be a malonate-activating enzyme in the nodule and also in bacteroids. However, nothing is known about the fate of malonate in nodule or in bacteroids. Here we describe the purification and properties of a novel malonyl-CoA synthetase from *R. japonicum*. This enzyme is distinct from *Pseudomonas* malonyl-CoA synthetase, since AMP and PP_i instead of ADP and P_i are the ATP-derived products.

MATERIALS AND METHODS

Materials

Rhizobium japonicum USDA 110 and *Glycine max* were obtained from the Korea Genetic Engineering Center, Daejeon, Korea. Affi-Gel Blue, hydroxyapatite, and Coomassie Brilliant Blue R-250 were purchased from Bio-Rad Laboratories. Q-Sepharose, Reactive Red-120-agarose, ATP, CoA, 3'-dephosphoCoA, Trizma base, pyruvate kinase/lactate dehydro-

genase and sodium malonate were purchased from Sigma. PEI (polyethyleneimine)-cellulose plates were from Schleicher und Schüll. [α -³²P]ATP and [γ -³²P]ATP were purchased from Amersham International.

Growth of bacteria

Rhizobium japonicum USDA 110 were grown in GYP medium, pH 6.8, containing 0.05% potassium phosphate, 0.02% MgSO₄·7H₂O, 1% glucose, 0.02% NaCl and 0.1% yeast extract at 30 °C with a vigorous shaking in 500-ml baffled flasks for 4 days. Glucose was separately sterilized and added to the medium.

Isolation of bacteroids

Bacteroids was isolated from nodules collected from 33-day-old soybean plants [8]. The presence of bacteroids was confirmed by measuring alanine dehydrogenase and malate dehydrogenase as marker enzymes [9].

Malonyl-CoA synthetase assay

Malonyl-CoA synthetase activity was assayed by the methods described previously [10]. Among the various assay methods, malonohydroxamate assay and direct spectrophotometric assay at 232 nm were routinely used for purification and characterization. Assay of the enzyme activity was possible by an AMP-myokinase coupling method, and this method was the most sensitive. The reaction mixtures contained 50 μ mol of Tris/HCl (pH 8.4), 1 μ mol of MgSO₄, 10 μ mol of malonate, 1 μ mol of ATP, 1 μ mol of phosphoenolpyruvate, 0.1 μ mol of NADH, 0.1 μ mol of coenzyme A, 5 μ l of 'PK/LDH' (3.5 units of pyruvate kinase and 5.0 units of lactate dehydrogenase), 1 μ l of myokinase (1.33 units), and enzyme in a final volume of 1.0 ml. The reaction was initiated by the addition of malonate, and the decrease in absorbance at 340 nm was measured on a spectrophotometer at 30 °C.

Abbreviation used: PEI, polyethyleneimine.

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Extraction of malonyl-CoA synthetase and Affi-Gel Blue chromatography

R. japonicum organisms (~31 g wet wt.), harvested and stored in a freezer, were thawed and resuspended in 75 ml of 20 mM-Tris/HCl buffer, pH 7.4, containing 1 mM-MgCl₂ and 15% glycerol. This cell suspension was cooled to 4 °C and sonicated. The sonicated mixture was centrifuged at 20000 g for 20 min. The pellet was resuspended in the same buffer, the extraction procedure was repeated twice, and the supernatants were pooled. To the combined supernatants 100 ml of Affi-Gel Blue suspension in the extraction buffer was added and stirred slowly at 4 °C for 2 h. Then the gel was packed in a column (3.6 cm × 10 cm) and washed with the same buffer. The absorbed proteins were eluted with a linear gradient of 0 to 1.5 M-NaCl in a total volume of 400 ml of the same Tris buffer. The fractions containing malonyl-CoA synthetase were pooled, and dialysed against 20 mM-Tris/HCl (pH 8.9)/15% glycerol. Then insoluble material was removed by centrifugation.

Anion-exchange chromatography on Q-Sepharose

The enzyme solution obtained from the previous step was subjected to a Q-Sepharose column (3.4 cm × 5.5 cm) pre-equilibrated with the same buffer for dialysis. After washing, the column with the same buffer, the proteins were eluted with a pH gradient (8.9–7.0) in a total volume of 300 ml. The fractions containing malonyl-CoA synthetase were pooled and dialysed against 20 mM-potassium phosphate (pH 6.9)/15% glycerol/10 mM-MgCl₂.

Affinity chromatography on reactive Red-120 agarose column

The enzyme solution obtained from the previous step was applied to a Red-120-agarose column (2.4 cm × 3 cm) and washed with the same buffer. The absorbed proteins were eluted with a linear gradient of 0–0.7 M-KCl in a total volume of 300 ml. The enzyme fractions were pooled and dialysed against 5 mM-potassium phosphate (pH 6.9)/15% glycerol.

Chromatography on a hydroxyapatite column

The enzyme solution obtained from the Red-agarose column was subjected to a hydroxyapatite column (2.4 cm × 3 cm) pre-equilibrated with the previous dialysis buffer. After washing the column with the buffer, the absorbed proteins were eluted with a linear gradient of 5–150 mM-potassium phosphate in a total volume of 300 ml. The fractions containing malonyl-CoA synthetase were pooled, concentrated, and dialysed against 20 mM-phosphate, pH 6.9 and 15% glycerol. This enzyme solution was stored at 4 °C and used for all subsequent experiments.

Electrophoresis

SDS/PAGE [11] was performed to determine the purity of the final preparation of the enzyme and to determine subunit M_r of malonyl-CoA synthetase. The polyacrylamide concentration of running gel was 12%, and the stacking gel was 3%. Marker proteins were phosphorylase *b* (M_r 94000), BSA (67000), ovalbumin (43000), carbonic anhydrase (30000), soybean trypsin inhibitor (20100) and α -lactalbumin (14400).

Determination of M_r

Molecular weight of malonyl-CoA synthetase was estimated by gel filtration on a Sephacryl S-300 column (1.5 cm × 90 cm). The column was calibrated with Blue Dextran and standard proteins: catalase (M_r 244000), γ -globulin (150000), BSA, ovalbumin and myoglobin (17200).

Determination of pI

The pI of malonyl-CoA synthetase was determined by using a

Phast Gel isoelectric-focusing (pI 3–9) system (Pharmacia). Reference proteins (pI) were lentil lectin [basic (8.66), middle (8.45) and acidic (8.15)], horse myoglobin [basic (7.86)], horse myoglobin [acidic (6.85)], human carbonic anhydrase B (6.55), bovine carbonic anhydrase B (5.85), β -lactoglobulin A (5.20), soybean trypsin inhibitor (4.58) and amyloglucosidase (3.50).

Product identification

Malonyl-CoA formed by the enzyme reaction was identified as its hydroxamic acid derivative by paper chromatography with *n*-butanol/ethanol/water (2:2:1, by vol.) [12]. The increase in thioester bond due to the formation of malonyl-CoA was monitored directly at 232 nm. AMP and PP_i were identified on PEI-cellulose plates with developing solvent of 1.2 M-NaCl or 0.7 M-sodium phosphate buffer, pH 3.5. In addition to this, AMP formation was also determined spectrophotometrically at 340 nm using the AMP-myokinase coupled assay.

Amino acid sequence analysis

Amino acid sequence analysis was performed on an Applied Biosystems model-471A protein/peptide sequencer equipped with an on-line phenylthiohydantoin analyser.

RESULTS

Malonyl-CoA synthetase in nodule bacteroid and free-living cells of *R. japonicum*

Malonyl-CoA synthetase activity was measured in a crude cell-free extract of *R. japonicum* bacteroid isolated from 33-day-old soybean nodule. The specific activity was 23.1 nmol/min per mg. In order to determine whether this enzyme is constitutive or inducible, the enzyme activity was measured in cell-free extracts of *R. japonicum* grown on different carbon sources. The specific activities (in nmol/min per mg) of the enzyme in the extract of cells grown on malonate, succinate, acetate, glucose, lactose, glycerol and histidine, were 11.1, 8.66, 15.4, 16.3, 16.7, 15.4 and 19.9 respectively. These results indicate that malonyl-CoA synthetase is expressed constitutively in the cells grown on a variety of carbon sources. The enzyme activity was also found in a cell-free extract of *R. trifolii*, but not in those of other symbiotic nitrogen-fixing bacteria such as *R. meliloti*, *A. tumefaciens* and *K. pneumoniae* (results not shown).

Purification of malonyl-CoA synthetase

In order to characterize such a constitutive malonyl-CoA synthetase, the enzyme was purified from the cell-free crude extracts prepared from the cells grown on GYP medium. By a batch treatment of this crude extract with Affi-gel Blue, malonyl-CoA synthetase was absorbed on the Blue gel, but the bulk of the other proteins stayed in the supernatant. Ion-exchange chromatography of the enzyme preparation on Q-Sepharose revealed that the enzyme activity appeared in the fractions collected between the second and third protein peaks. Considerable purification was achieved by this ion-exchange and by Red-agarose chromatography. Subsequent chromatography of the enzyme preparation on hydroxyapatite showed three eluted protein peaks. The enzyme activity was determined in the fractions of the main protein peak, and at least two-thirds of the protein collected from the leading shoulder was electrophoretically homogeneous malonyl-CoA synthetase (Fig. 1). In any case, the combination of these purification methods achieved nearly a 2740-fold purification of malonyl-CoA synthetase with an overall recovery of about 38.6% (Table 1).

Criteria of purity

Malonyl-CoA synthetase eluted from the hydroxyapatite

Fig. 1. Chromatographic purification of malonyl-CoA synthetase from *Rhizobium japonicum*
(a) Affinity chromatography with Affi-gel Blue; (b) anion-exchange chromatography with Q-Sepharose; (c) affinity chromatography with reactive Red-120-agarose; (d) chromatography with hydroxyapatite. For details, see the text.

Table 1. Purification of malonyl-CoA synthetase from *Rhizobium japonicum*

Enzyme activity was measured by the hydroxamate assay method.

Stage of purification	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min per mg)	Recovery (%)	Purification (-fold)
Crude extract	1779.6	14788.4	8.31	100	1.0
Affi-gel Blue	33.4	13273.1	397.4	89.7	47.8
Q-Sepharose	2.76	12360.9	4478.6	83.5	538.9
Red-agarose	0.918	9480.9	10327.8	64.1	1242.8
Hydroxyapatite	0.250	5692.5	22770.0	38.6	2740

column was homogeneous. PAGE of the purified enzyme showed a protein band which was coincident with the band by activity staining (results not shown). SDS/PAGE showed only one protein band upon staining with Coomassie Blue (Fig. 2). Gel filtration of the enzyme preparation on Sephacryl S-300 showed a symmetrical peak of protein (M_r 58000) which was coincident with the enzyme activity profile (results not shown).

Product identification

Since the enzyme catalyses the formation of malonyl-CoA directly from malonate and CoA in the presence of ATP and Mg^{2+} , the reaction products are estimated to be malonyl-CoA, ADP and P_i , or malonyl-CoA, AMP and PP_i . The formation of thioester bond of malonyl-CoA was measured directly by following the increase in absorbance at 232 nm or by converting the thioester to a hydroxamate. The time-dependent increase in difference spectra at 232 nm suggested the formation of a thioester

Fig. 2. SDS/PAGE of purified malonyl-CoA synthetase
Size markers are indicated.

Fig. 3. Autoradiogram of t.l.c. analysis of reaction products

(a) T.l.c. analysis of reaction products in the presence of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ with 1.2 M-NaCl as a developing solvent. (b) and (c), with 0.7 M-sodium phosphate buffer, pH 3.5, as a developing solvent, t.l.c. analysis of the reaction products in the presence of $[\beta\text{-}^{32}\text{P}]\text{ATP}$ and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, respectively. This developing solvent is good for the separation of PP_i from ATP. $[\beta\text{-}^{32}\text{P}]\text{ATP}$ was prepared by the phosphorylation of AMP by myokinase with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, followed by the phosphorylation of $[\beta\text{-}^{32}\text{P}]\text{ADP}$ by acetate kinase reaction. $[\beta\text{-}^{32}\text{P}]\text{ADP}$ and $[\beta\text{-}^{32}\text{P}]\text{ATP}$ at each step was purified on a DEAE-Sephacel ion-exchange column using a 0.1–0.4 M linear gradient of triethylamine bicarbonate [17,18].

bond. The formation of malonyl-CoA was also confirmed by the identification of malonohydroxamate (R_f 0.19) using paper chromatography. The nucleotide product was measured by a coupled enzymic assay and directly by t.l.c. The decrease in absorbance at 340 nm was monitored as soon as myokinase was added in the reaction mixture containing the ADP coupling assay reagents, indicating the formation of AMP instead of ADP. This result raised another question, whether AMP/ PP_i were products or AMP/ 2P_i were products through a cryptopyrophosphoryl transfer. Therefore the enzyme reactions were carried out in the presence of $[\alpha\text{-}^{32}\text{P}]$, $[\beta\text{-}^{32}\text{P}]$ or $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and the reaction products were analysed on PEI cellulose plates with developing solvents 1.2 M-NaCl or 0.7 M-sodium phosphate buffer, pH 3.5. As shown in Fig. 3, only labelled AMP and PP_i in the reaction mixtures containing $^{32}\text{P}]\text{ATP}$ were identified, indicating that AMP/ PP_i , but not AMP/ 2P_i , were the products.

Properties of malonyl-CoA synthetase

M_r and pI. The M_r of malonyl-CoA synthetase was estimated to be 58000 by gel filtration on a calibrated column of Sephacryl S-300. SDS/PAGE of the enzyme (Fig. 2) revealed only one protein band with an M_r of 58000, indicating that this malonyl-CoA synthetase is a monomeric enzyme composed of only one polypeptide. The enzyme from *P. fluorescens*, however, is a dimeric enzyme composed of two non-identical subunits. A similar synthetase, succinyl-CoA synthetase, is also multimeric. The pI of the native malonyl-CoA synthetase was estimated to be 7.3 by a gel-isoelectric-focusing method with standard pI markers.

N-Terminal heterogeneity. The amino acid sequence of the N-terminal part of the purified enzyme was determined by a microsequence analyser to be:

Met-Asn-Arg-Ala-Ala-Asn-Ala-Asn-Leu-Phe-

However, the preparation also contained a protein missing N-terminal methionine residue in about 40% of the total protein. At present it is not clear whether the N-terminal heterogeneity is related to the enzyme activity.

pH-dependence. As the pH increased from 6.5 to 7.9 there was a dramatic increase in the enzyme activity, and subsequent increase in pH, caused a fairly rapid decline in the activity, resulting in a rather sharp optimum pH around 7.9. This optimum pH was slightly higher than that (pH 7.5) for malonyl-CoA synthetase from *P. fluorescens*.

Effect of glycerol. The enzyme preparation contained 15% glycerol to maintain stability. Its effect on the enzyme activity was investigated because glycerol alters the substrate specificity or affinity of some enzymes [13,14]. When the glycerol content in the assay mixture was 1.24–3.24% (v/v), enzyme activity was highest. Increased or decreased glycerol content gradually decreased activity. Therefore most enzyme assays were performed in a buffer containing about 2% glycerol.

Substrate specificity. The substrate specificity of purified malonyl-CoA synthetase was determined with substrate, substrate analogues and metabolic intermediates such as malonate, 2-methylmalonate, acetate, oxalate, succinate, isocitrate, citrate, malate, aspartate, glutarate, CoA, 3'-dephosphoCoA, ATP, ADP, AMP, CTP and GTP. When methylmalonate, acetate or dephosphoCoA was used as substrate instead of malonate, methylmalonyl-CoA or acetyl-CoA or malonyl-dephosphoCoA was synthesized at a rate of 53.6 or 6.4 or 22.5% respectively relative to the formation of malonyl-CoA (Table 2). However, no other compounds examined were converted into any thioester compound, indicating a rigid substrate specificity. No nucleotides except ATP supported catalysis. High substrate specificity was also observed in the enzyme from *P. fluorescens* [1]. Measurement of the reverse reaction failed. That is, measurement of the

Table 2. Substrate specificity of malonyl-CoA synthetase

In order to determine the substrate specificity, the formation of the thioester bond was monitored spectrophotometrically at 232 nm in the presence of substrate or substrate analogues.

Substrates and their analogues	Activity (nmol/min)*	Relative rate (% of control)
Malonate	20.7	100
2-Methylmalonate	11.1	53.6
Acetate	1.32	6.4
Oxalate	ND	0.0
Succinate	ND	0.0
CoA	20.7	100
3'-DephosphoCoA	4.65	22.5
ATP	20.7	100
GTP	ND	0.0
CTP	ND	0.0
ADP	ND	0.0
AMP	ND	0.0

* ND = not detectable.

degradation of malonyl-CoA in the presence of the enzyme solution containing AMP, PP_i , P_i and Mg^{2+} was attempted spectrophotometrically at 232 nm. But no change at 232 nm was observed under the various conditions, indicating that the reverse reaction is negligible.

Ion requirement. This enzyme required Mg^{2+} for catalysis. The K_m for MgSO_4 or MgCl_2 was $13.7 \mu\text{M}$. Among a variety of other cations such as Mn^{2+} , Zn^{2+} , Ca^{2+} , Li^+ , Na^+ , K^+ , and NH_4^+ , only Mn^{2+} could substitute for Mg^{2+} without any activity difference. Anions such as SO_4^{2-} and Cl^- did not affect the enzyme activity.

Effects of substrate concentration. With increasing concentration of substrates, the rate of malonyl-CoA formation was increased and typical Michaelis-Menten-type substrate-saturation patterns were obtained. The double-reciprocal plots were linear, and from such plots K_m and $V_{\max}$ values were calculated to be $200 \mu\text{M}$ and $21.3 \mu\text{mol/min per mg}$ for malonate, $33.3 \mu\text{M}$ and $29.4 \mu\text{mol/min per mg}$ for ATP, $87.0 \mu\text{M}$ and $41.7 \mu\text{mol/min per mg}$ for CoA respectively. These kinetic constants are much lower than those obtained by the *Pseudomonas* enzyme.

Non-competitive inhibition by succinate. Succinate is a structural analogue of malonate and a major carbon source for bacteroids. Therefore the effect of succinate on the malonyl-CoA synthetase activity was determined. Succinate inhibited the enzyme activity non-competitively (Fig. 4). The K_i was 47.3 mM .

Effect of AMP and ADP. AMP is one of the products for this enzyme catalysis. However, when the enzyme activity was determined by measuring AMP with the AMP myokinase coupled assay method, enzyme activity about 3.5 times higher than that by other methods was always determined. This suggests that AMP concentration may influence the enzyme activity. With respect to ATP, AMP, as expected, inhibited competitively the enzyme with a K_i of $55 \mu\text{M}$. ADP also inhibited the enzyme competitively with respect to ATP but K_i was $250 \mu\text{M}$, which is nearly fivefold higher than that for AMP (Fig. 5).

Effect of inhibitors. Malonyl-CoA synthetase was severely inhibited by diethylpyrocarbonate and pyridoxal-5'-phosphate,

Fig. 4. Effect of succinate on malonyl-CoA formation

A typical Michaelis-Menten substrate saturation pattern was obtained. The double-reciprocal plots were linear and, from such plots, K_i was calculated.

Fig. 5. Effect of AMP and ADP on malonyl-CoA formation

Typical Michaelis-Menten substrate saturation patterns were obtained. The double reciprocal plots were linear and K_i values were calculated from such plots.

whereas iodoacetamide, *p*-chloromercuriphenylsulphonate, *N*-acetylimidazole, and phenylglyoxal did not show measurable inhibition. When enzyme was treated with diethylpyrocarbonate (2 mM) for 7 min and with pyridoxal-5'-phosphate at 3 mM for 15 min respectively, the enzyme activity almost completely disappeared. These results indicate that the imidazole group of the histidine residue and the amine group of the lysine residue of the enzyme may play important roles in enzyme catalysis.

Stability. The purified enzyme in 20 mM-phosphate buffer (pH 6.9)/15% glycerol at 4°C showed no detectable loss of enzyme activity for several months. At 50°C , the enzyme lost 80% of activity within 30 min. But when ATP and malonate were added to the enzyme solution, activity loss at 50°C was reduced down to 50%. When ATP, malonate, CoA, ATP+CoA or malonate+CoA respectively were added to the enzyme, no protection was observed.

DISCUSSION

In this study a novel malonyl-CoA synthetase, which catalyses the formation of malonyl-CoA directly from malonate and CoA in conjunction with the hydrolysis of ATP to AMP and PP_i , was detected and characterized in bacteroids of soybean nodules,

purified from *R. japonicum*. This enzyme is a typical AMP/PP_i-forming ligase which shows high specificity towards malonate. ADP/P_i-forming malonyl-CoA synthetase has been reported in rat brain and isolated from malonate-grown *P. fluorescens* [1]. The *Rhizobium* enzyme shows distinct differences from the inducible *Pseudomonas* enzyme. The *Rhizobium* enzyme is monomeric and its *M_r* is 58 000, whereas the *Pseudomonas* enzyme is dimeric. But the α -subunit (*M_r* 68 000) of the *Pseudomonas* enzyme has been suggested to be a catalytic subunit which has an essential imidazole group of the histidine residue and an amine group. It would be of interest to know whether the *Rhizobium* enzyme also has essential catalytic groups modified by diethylpyrocarbonate and pyridoxal-5'-phosphate. These results suggest that the enzymes from the two different bacteria may have a common structure in their catalytic sites. At present the catalytic mechanism has not been studied, and so it is not clear if any intermediate, either malonyl adenylate or malonyl pyrophosphate, is formed during *Rhizobium* malonyl-CoA synthetase catalysis. However, a concerted catalytic mechanism cannot be ruled out in consideration of the fact that malonohydroxamate in the reaction mixture, including hydroxylamine but excluding CoA, has not been detected.

Malonate-specific enzyme has long been expected to exist in nodules, since free malonate has been known to occur in legumes and its level is known to increase under symbiotic conditions [15]. It has also been reported that, in the symbiotic host-plant cell, malonate is passively transported into bacteroids [16]. But nothing is known about the fate of malonate in bacteroids. The finding of malonyl-CoA synthetase in soybean bacteroids, the purification of the enzyme from *R. japonicum*, and the ensuing characterization clearly show that a malonate-metabolizing en-

zyme exists in bacteroids and that the enzyme may play a role in symbiosis.

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