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Identification of Malonate-specific Enzymes, Malonyl-CoA Synthetase and Malonamidase, in *Rhizobia*

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*Rhizobia*에서 Malonyl-CoA Synthetase와 Malonamidase의 확인

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ABSTRACT: Two malonate-specific enzymes, malonyl-CoA synthetase and malonamidase, were found in free-living cultures of *Rhizobium japonicum*, *Rhizobium meliloti*, and *Rhizobium trifolii*, that infect plant roots where contain a high concentration of malonate. Malonyl-CoA synthetase catalyzes the formation of malonyl-CoA, AMP, and PPi directly from malonate, coenzyme A, and ATP in the presence of Mg^{2+}. Malonamidase is a novel enzyme that catalyzes hydrolysis and malonyl transfer of malonamate, and forms malonohydroxamate from malonate and hydroxylamine. Both enzymes are highly specific for malonate. These results show that *Rhizobia* have enzymes able to metabolize malonate and suggest that malonate may be used in symbiotic carbon and nitrogen metabolism.

KEY WORDS□Malonyl-CoA synthetase, malonamidase, *Rhizobia*

Malonate is present in high concentration in the tissues of various plants and especially in many legumes (Bentley, 1952). In soybeans, malonate is the most abundant organic acid in leaf and root tissue, whereas fumarate is predominant in the stem (Stumpf and Burris, 1981). In nodules from 33-day-old soybean, malonate is also predominant. However, the malonate concentration in root and nodule tissue of 33-day-old soybeans is depressed after the addition of either nitrate or ammonia. Malonate at its nodule concentration has been shown to be transported to bacteroids by a passive mechanism (Reibach and Streeter, 1984). These results suggest that there should be a malonate metabolizing system in symbiotic bacteria if only because malonate is a competitive inhibitor of succinate dehydrogenase, an enzyme implicated in bacteroid carbon metabolism. However, nothing is known about the fate of malonate in bacteroids. In fact, very little is known about malonate-specific enzymes in any bacteria. Malonyl-CoA synthetase from *Pseudomonas fluorescens* is the only enzyme known to convert free malonate to malonyl-CoA(Kim and Bang, 1985; Kim and Lee, 1986).

In this paper we present evidence that two novel malonate specific enzymes, malonyl-CoA synthetase and malonamidase, occur in free-living *Rhizobia*.

MATERIALS AND METHODS

Strains

Rhizobium japonicum USDA 110, *Rhizobium meliloti* 1021, and *Rhizobium trifolii ATCC* 14479, were obtained from Korea Genetic Engineering

Center.

Materials

Sodium malonate, ATP, CoA, diethylpyrocarbonate (DEP), and pyridoxal-5′-phosphate(PLP) were purchased from Sigma Chem. Co., U.S.A. Adenylate kinase, pyruvate kinase, and lactate dehydrogenase were also purchased from Sigma Chem. Co.

Synthesis of malonamic acid

Dried ammonia gas was bubbled in 10 m*l* of absolute methanol at 0℃, 1.1 g of monomethyl malonate was then added dropwise and the mixture was kept in the refrigerator for about 4 days. After evaporation of methanol, the reaction mixture was diluted with 6N HCl and extracted with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. A small amount of dried CH_2Cl_2 was added to the concentrate and malonamic acid was crystallized. The crystals were washed twice with CH_2Cl_2 and produced white, needle-shaped crystals: 350 mg (36%), mp; 122-123℃, ^1H NMR 3.35(s2H, 6.98-7.89(br, 1H)).

Culture media

Mal-medium (pH 6.8) contained 10g of malonate, 3g of NH_4Cl, 2g of KH_2PO_4, 0.4g of $MgSO_46H_2O$, and 10mg of $FeSO_47H_2O$ per liter. Modified Brown's medium (pH 7.0) contained 3g of malonate, 9.72g of HEPES, 0.25g of $MgSO_47H_2O$, 0.2g of NaCl, 0.02g of $CaCl_2$, 0.2g NH_4Cl, and 10 m*l* of trace elements solution per liter. The trace elements solution contained 6.6 mg of $FeCl_36H_2O$, 15 mg of $EDTA_2Na$, 0.16 mg of $ZnSO_47H_2O$, 0.2 mg of Na_2MoO_4, 0.25 mg of H_3BO_3, 0.2 mg of $MnSO_45H_2O$, 0.02 mg of $CuSO_4$, 1 μg of $CoCl_2$, 1 mg of thiamine, 2 mg of calcium panthothenate, and 1 μg of biotin per liter. YM-medium contained 10g of mannitol, 1g of yeat extract, 0.5g of KH_2Po_4, 0.2g of $MgSO_47H_2O$, and 0.2g of NaCl per liter. GYP-medium is YM-medium in which mannitol was substituted by glucose. The *Rhizobia* were cultured in a shaking incubator at 30℃,

Malonyl-CoA synthetase assay

Malonyl-CoA synthetase catalyzes the formation of malonyl-CoA, AMP, and PPi directly from malonate and coenzyme A in the presence of ATP and Mg^{2+}:

$$\text{Malonate} + \text{CoA} + \text{ATP} \rightarrow \text{Malonyl} - \text{CoA} + \text{AMP} + \text{PPi}$$

The enzyme activity was determined by measuring the formation of either malonyl-CoA or AMP. Malonyl-CoA formed was either determined by measuring the increase in absorbance at 232 nm by the formation of the thioester bond of malonyl-CoA or by converting malonyl-CoA of malonohydroxamate, a compound that forms colored complex with Fe^{3+} (Kim and Bang, 1988). The formation of AMP was determined by measuring the decrease in absorbance at 340 nm due to the disappearance of NADH using the coupled reactions shown below:

$$\text{AMP} + \text{ATP} - \text{I} \rightarrow 2\text{ADP}$$

$$\text{ADP} + \text{PEP} + \text{H}^+ - \text{II} \rightarrow \text{pyruvate} + \text{ATP}$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \rightleftarrows \text{III} \rightleftarrows \text{lactate} + \text{NAD}^+.$$

I, adenylate kinase: II, pyruvate kinase: III, lactate dehydrogenase

The reaction mixture contained 50 mM Tris-HCl buffer, pH 7.9; 10 mM sodium malonate; 1 mM $MgSO_4$; 1 mM PEP; 1 mM ATP; 0.1 mM CoA; 0.1 mM NADH; adenylate kinase, 1.33 units in 1 μ*l*; pyruvate kinase/lactate dehydrogenase, 3.5 /5.0 units in 5 μ*l*; and enzyme and water in a final volume of 1 m*l*. The reaction was initiated by the addition of sodium malonate and the decrease in absorbance at 340 nm was monitored by a spectrophotometer. The molar extinction coefficient of NADH is 6220 $M^{-1}cm^{-1}$.

Malonamidase assay

Malonamidase catalyzes the hydrolysis of malonamate and the formation of malonohydroxamate from malonate and hydroxylamine.

$$\text{Malonamate} + \text{H}_2\text{O} \rightarrow \text{malonate} + \text{NH}_3$$

$$\text{Malonate} + \text{NH}_2\text{OH} \rightarrow \text{malonohydroxamate}$$

The enzyme activity was determined by three different methods.

Malonohydroxamate assay method-1. This is based on the malonyl transfer reaction from malonamate to hydroxylamine. The reaction mixture contained 100 mM MOPS, pH 6.8; 10 mM malonamate; 200 mM NH_2OH; and enzyme and water in a final volume of 0.5 m*l*. After the reaction the malonohydroxamate formed was determined as described above.

Malonohydroxamate assay method-2. This is based on the formation reaction of malonohydroxamate from malonate and NH_2OH by this enzyme. The reaction mixture contained 100 mM MOPS, pH 6.8; 40 mM sodium

malonate; and 200 mM NH_2OH; and water in a final volume of 0.5 m*l*. The malonohydroxamate generated by this method was determined as described above.

NH_4^+ assay method. This is based on the determination of ammonia generated from the hydrolysis of malonamate. The reaction mixture contained 100 mM MOPS, pH 6.8; 10 mM malonamate; and enzyme and water in a final volume of 0.5 m*l*. After incubation, aliquots were taken and diluted with water to 0.5 m*l*. Then 0.5 m*l* of phenol nitroprusside solution was added to the sample and mixed gently, followed by the addition of 0.5 m*l* of sodium hypochlorite solution (Weatherburn, 1967). After sitting, the color of the mixture was measured by a spectrophotometer at 640 nm. The molar extinction coefficient of indophenol produced by ammonium ion is $1.74\times 10^4 M^{-1}\ cm^{-1}$.

Preparation of crude extract and partial purification of the enzymes

R. japonicum enzymes were purified by the following procedure. 31 g (wet wt.) of cells grown on GYP-medium were harvested, resuspended in 20 mM Tris−HCl buffer, pH 7.4, and then broken by sonication. The crude cell extracts were prepared by a centrifugation of the preparation at 20,000g for 20 min.

R. japonicum enzymes were purified by the following procedure. Affi-gel blue was directly added to the crude extract (1703 mg protein in 130 m*l*) and stirred at 4℃ for 2 h. The gel suspension was then packed in a column (3.6×10 cm), and washed with the same buffer. Malonyl-CoA synthetase was bound to the column but not malonamidase. The proteins bound on the column were eluted by a linear gradient of 0 to 1.5 M NaCl and malonyl-CoA synthetase was eluted at 0.4 M NaCl. The specific activity of this malonyl-CoA synthetase preparation was 85.3 nmol/min/mg. Malonamidase that flowed through the Affi-gel blue column was further purified by ion exchange chromatography. The protein fractions were applied to a DEAE Sephacel column (3.4×3.5 cm) pre-equilibrate with 20 mM Tris - HCl buffer, pH 7.4, and the proteins on the column were eluted with a linear gradient of 0 to 1 M NaCl. The major peak of malonamidase was eluted at 0.28 M NaCl and then was a minor peak at 0.36 M NaCl. The malonamidase (specific activity, 202 nmol/min/mg) which appeared on the main peak was used for further characterization.

R. trifolii malonyl-CoA synthetase was purified by an Affi-gel blue column. The crude extract (77.7 mg protein in 20 m*l*) was applied on Affi-gel blue column (1.5×4.5 cm) pre-equilibrated with the same 20 mM Tris-HCl buffer, pH 7.4. Malonyl-CoA synthetase (specific activity, 3546 nmol/min/mg) was eluted at 0.26 M NaCl by a linear gradient of 0 to 1 M NaCl.

R. meliloti malonamidase was purified by ion exchange chromatography. The crude extract(94 mg in 20 m*l*) was loaded on a DEAE Sephacel column (1.5×4.5 cm) pre-equilibrated with the same Tris-HCl buffer, and the bound proteins were eluted by a linear gradient of 0 to 0.5 M NaCl. Malonamidase was eluted at 0.33 M NaCl. This preparation of the enzyme (specific activity, 144.4 nmol/min/mg) was used for further characterization.

RESULTS AND DISCUSSION

Malonyl-CoA synthetase and malonamidase activity in the crude extracts.

All three bacteria grew on GYP-medium and YM-medium. However, *R. japonicum* and *R. trifolii* did not grow on Mal-medium which contained malonate as the sole source of carbon, whereas *R. meliloti* grew well on this medium. However, *R. japonicum* and *R. trifolii* would grow on Brown's malonate medium. Enzyme activities were determined by the malonohydroxamate method. As shwon in Table 1, malonyl-CoA synthetase and malonamidase activities were found in crude extracts of *R. japonicum* grown on three different media. The specific activities were not very different in the presence of malonate, indicating that these enzymes may not be inducible. *R. meliloti* contained malonamidase activity but no malonyl-CoA synthetase, whereas *R. trifolii* contained malonyl-CoA synthetase but not malonamidase. Malonamidase in *R. meliloti* and malonyl-CoA synthetase in *R. trifolii* also do not seem to be inducible. The malonyl-CoA synthetase content in *R. trifolii* was at least 16 times more than that in *R. japonicum*. The activities of the enzymes were also determined by assay methods other than the malonohydroxamate method. In all cases, the activities were linearly correlated with the reaction time and the amount of enzymes.

Substrate specificity

Substrate specificity of the purified enzymes

Table 1. *Bacterial growth on different media and the enzyme activity in the crude extracts of the bacteria. Specific activity was determined by malonohydroxamate assay method. Bacterial growth: −; very poor, +; well. MS; malonyl-CoA synthetase. MA; malonamidase. ND; Not detectable. *Data obtained from the cells grown on a modified Brown's malonate media.*

Media/Enzyme (nmol/min/mg)	Mal-med		GYP-med		YM-med	
Bacteria	MS	MA	MS	MA	MS	MA
R. japonicum	−		+		+	
	11.1*	16.5*	8.3	24.4	12.7	17.0
R. meliloti	+		+		+	
	ND	12.6	ND	19.1	ND	23.2
R. trifolii	−		+		+	
	179.8*	ND	215.4	ND	201.9	ND

was determined by using mono-, di-, and tricarboxylic acids and two amino acids instead of malonate in the reaction mixtures. As shown in Table 2, even though some amidase activity was determined with succinate and malate, the substrate specificity on malonate was extremely high. Further purification of malonamidase will exclude the nonspecific amidase activity with succinate and malate.

PARTIAL CHARACTERIZATION OF MALONYL-CoA SYNTHETASE

Product identification

The formation of malonyl-CoA in the reaction mixtures was determined by two different methods. Fig. 1 shows the time-dependent increase in UV absorption, clearly indicating that there is an enzyme catalyzing the formation of the thioester bond directly from malonate and coenzyme A in the presence of ATP. The UV pattern showing maximum absorption at 232 nm was almost identical for two reaction mixtures, one with *R. japonicum* enzyme and the other with *R. trifolii* enzyme. When the enzyme reaction was carried out in the presence of hydroxylamine and the product was analyzed by paper chromatography, there was a spot at R_f of 0.19, which is identical to that of a malonohydroxamate standard. In reactions with enzymes from two different sources, no hydroxamate other than malonohydroxamate was detected.

Table 2. *Substrate specificity expressed as % for malonate. The formation of the thioester bond for synthetase was determined by measuring the increase in absorbance at 232 nm. For amidase, the formation of hydroxamate with a variety of acids was determined by the malonohydroxamate assay method described in the text. R. jap.; Rhizobium japonicum. R. mel.; Rhizobium meliloti. R. tri.; Rhizobium trifolii. MS; malonyl-CoA synthetase. MA; malonamidase. *No activity was determined.*

Bacteria/ Enzymes	*R. jap.*		*R. mel.*		*R. tri.*	
Substrate	MS	MA	MS*	MA	MS	MA*
Malonate	100	100		100	100	
Acetate	6	0		0	2.7	
Oxalate	0	3		15	0	
Succinate	0	17		0	5.5	
Malate	0	34		7	0	
Citrate	0	9		13.3	0	
DL-isocitrate	0	12		10.3	0	
Aspartate	0	0		6.6	3.9	
Glutamate	0	0		0	0	

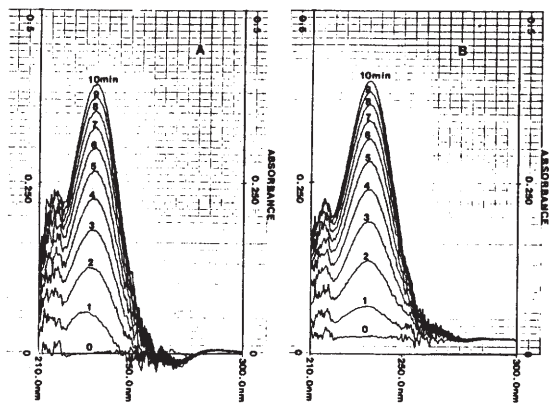

Fig. 1. *UV absorption spectra of malonyl-CoA synthetase reaction mixture.*
A; *R. japonicum* malonyl-CoA synthetase reaction. B; *R. trifolii* malonyl-CoA synthetase reaction. Increase in absorbance at 232 nm indicates increase in the formation of thioester bond of malonyl-CoA.

In many ATP dependent biochemical reactions, ADP/Pi or AMP/PPi or very rarely AMP/2Pi (Cooper and Kornberg, 1974; Milner and Wood, 1972) are produced. In malonyl-CoA synthetase catalysis, AMP was the product. As shown in Fig. 2, when the ADP coupling system, containing

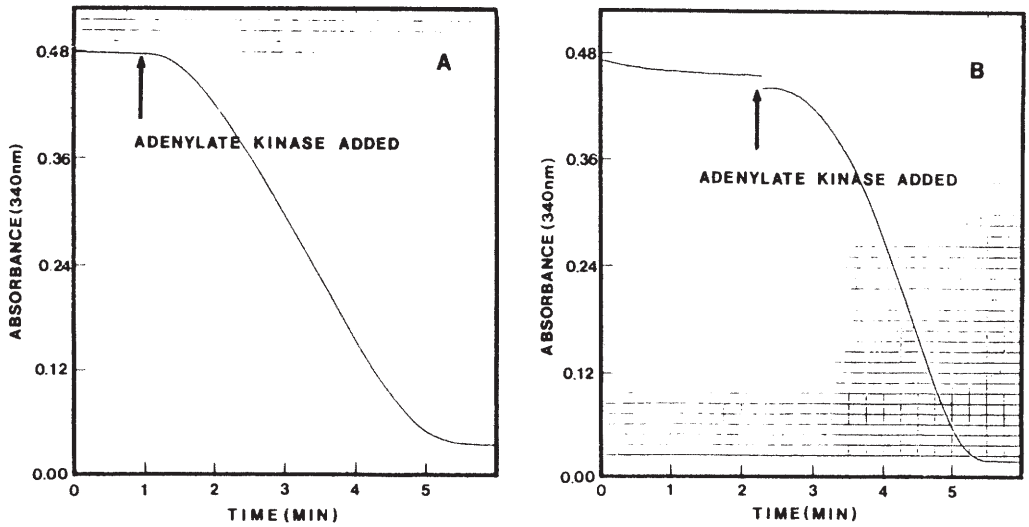

Fig. 2. *Decrease in absorbance at 340 nm by the addition of adenylate kinase to malonyl-CoA synthetase/ADP coupling reaction mixture.*
A; *R. japonicum* enyme. B; *R. trifolii* enzyme. The reaction mixture contained the complete malonyl-CoA synthetase reaction system containing enzyme, ATP, malonate, and CoA, and ADP coupling system containing PEP, NADH, pyruvate kinase, and lactate dehydrogenase. Decrease in absorbance at 340 nm by addition of adenylate kinase indicates the formation of ADP from AMP produced by malonyl-CoA synthetase.

PEP, pyruvate kinase, NADH, and lactate dehydrogenase, was added to the malonyl-CoA synthetase reaction mixture, no decrease in absorbance at 340 nm was monitored. But the decrease in absorbance at 340 nm was initiated as soon as adenylate kinase was added, indicating that AMP instead of ADP was formed. AMP formation as a product was monitored in the reaction mixtures containing malonyl-CoA synthetase from two different sources. AMP and PPi formation was also determined by TLC analysis of the reaction mixture in the presence of [α-^{32}P] ATP or [γ-^{32}P] ATP. For the TLC analysis, PEI cellulose plate with two different developing solvents were used. The developing solvent (1.2 M NaCl solution) for A plate (Fig. 3, A) was good for the separation of AMP from ADP, ATP, and Pi but it was not good for the separation of PPi from ATP. Therefore 0.7 M sodium phosphate buffer, pH 3.5, was used for the separation of PPi from ATP (Fig. 3, B). The autoradiogram of the TLC analysis clearly showed the two products, AMP and PPi, by the catalysis of malonyl-CoA synthetase from two different *Rhizobia*.

Kinetic constants

With increasing concentration of substrates, the initial rate of malonyl-CoA formation by *R. japonicum* and *R. trifolii* synthetase increased and typical Michaelis-Menten type substrate saturation patterns were obtained. The double reciprocal plots were linear, and from such plots, K_ms were calculated as shwon in Table 3.

The K_mS for ATP and for CoA were almost identical for the enzymes from two different sources, but the K_m for malonate was considerably different. The K_m for the *R. japonicum* synthetase (2.7 mM) was about one fifth of the K_m the *R. trifolii* enzyme (14.8 mM), indicating that *R. japonicum* enzyme had a high affinity for malonate. However, it is of interest to know that *R. trifolii* contains a higher activity of the synthetase than *R. japonicum* as described above.

Inhibition of *R. trifolii* malonyl-CoA synthetase by ATP

ATP is a substrate for malonyl-CoA synthetase. But with increasing concentration of ATP, the initial rate of malonyl-CoA formation by *R. trifolii* synthetase was linear up to 5 mM and then the rate was drastically decreased, showing a typical substrate inhibition in enzyme catalysis (Fig. 4).

pH optimum and metal ion requirement

Malonyl-CoA synthetase from *R. japonicum* showed its maximal activity at about pH 7.9 in 100 mM Tris-HCl buffer, whereas the enzyme

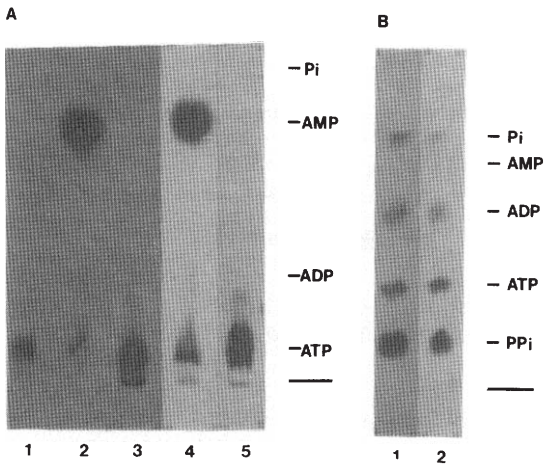

Fig. 3. *Autoradiogram of TLC analysis of malonyl-CoA synthetase reaction products.*
Plate A. PEI cellulose with 1.2 M NaCl as developing solvent was used. 1; [γ-^{32}P] ATP, 2; [^{32}P] labeled products from the *R. trifolii* malonyl-CoA synthetase reaction in the presence of [α-^{32}P] ATP, 3; [^{32}P] labeled products from the *R. trifolii* malonyl-CoA synthetase reaction in the presence of [γ-^{32}P] ATP, 4; [^{32}P] labeled products from the *R. japonicum* malonyl-CoA synthetase reaction in the presence of [α-^{32}P] ATP, 5; [^{32}P] labeled products from the *R. japonicum* malonyl-CoA synthetase reaction in the presence of [γ-^{32}P] ATP. Plate B. PEI cellulose wih 0.7 M sodium phosphate buffer, pH 3.5, as developing solvent was used. 1 and 2; [^{32}P] labeled products from *R. trifolii* and *R. japonicum* malonyl-CoA synthetase reactions in the presence of [γ-^{32}P] ATP, respectively.

Table 3. *Kinetic constants*
A. *Malonyl-CoA synthetase.* Enzyme activity was assayed by hydroxamate method.

Source of enzyme	K_m(mM) for		
	Malonate	ATP	CoA
R. japonicum	2.7	2	0.14
R. trifolii	14.8	2.1	0.11

B. *Malonamidase* Enzyme activity was assayed by hydroxamate method.

Source of enzyme	K_m(mM) for	
	Malonamate	Malonate
R. japonicum	1.62	16.2
R. meliloti	2.12	22.0

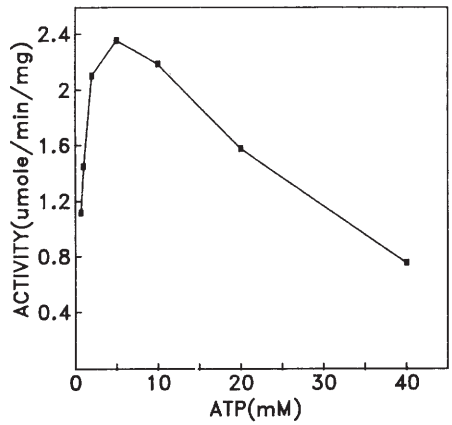

Fig. 4. *Effect of ATP concentration of R. trifolii malonyl-CoA synthetase catalysis.*

from *R. trifolii* showed it at about pH 7.1 in 100 mM potassium phosphate buffer. Both enzymes required a divalent metal ion for maximal activity, *R. Japonicum* synthetase was equally active with Mg^{2+} or Mn^{2+} but would accept only these metals. The *R. trifolii* synthetase had highest activity in the presence of Mg^{2+}, but showed about 90%, 40% and 3% activity even in the presence of Mn^{2+}, Zn^{2+} and Ca^{2+}, respectively.

Inactivation of malonyl-CoA synthetase by DEP and PLP

Malonyl-CoA synthetase from both sources was inactivated by DEP and PLP. When 9 nmole of *R. japonicum* synthetase was incubated with 2 mM DEP in potassium phosphate buffer, pH 6.9, complete inactivation was achieved within 7 min. Similarly, complete inactivation of 8.6 nmole *R. trifolii* enzyme in 1.5 mM DEP in potassium phosphate buffer, pH 7.1, was achieved within 5 min. These results indicate that a histidine residue in the enzyme may be essential for its activity. PLP inactivation on the enzyme was less sensitive than DEP inactivation. After incubation of the enzyme with PLP, the reactions were terminated by the incubation of the reaction mixture with $NaBH_4$ for 90 min. The incubation of 9 nmole *R. japonicum* enzyme with 3 mM PLP for 5 min achieved 70% inactivation, whereas the incubation of 7.1 nmole *R. trifolii* enzyme with 3 mM PLP for 5 min achieved only 46% inactivation.

PARTIAL CHARACTERIZATION OF MALONAMIDASE

Product identification

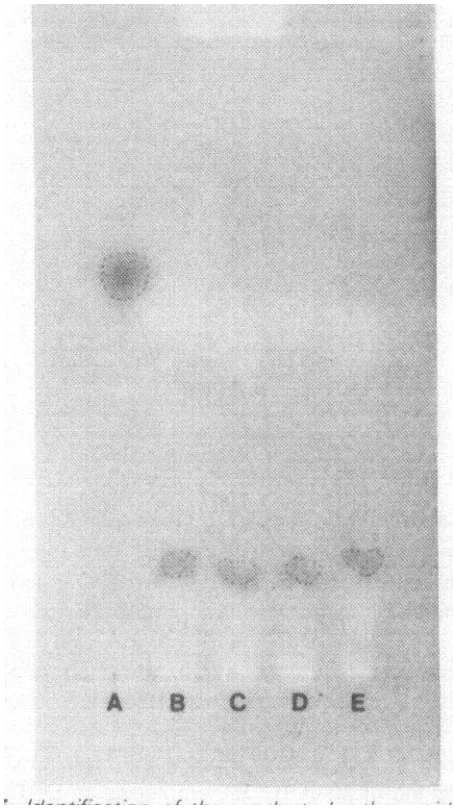

Fig. 5. *Identification of the products by the amidases catalysis with paper chromatography.* Developing solvent was a mixture of *n*-butanol, ethanol, and water (2:2:1). A: Authentic acetohydroxamate, B: Hydroxamate produced from malonamate and hydroxylamine by *R. japonicum* amidase, C: Hydroxamate produced from malonate and hydroxylamine by *R. japonicum* amidase, D: Hydroxamate produced from malonate and hydroxylamine by *R. meliloti* amidase, and E: Authentic malonohydroxamate.

When malonamate was incubated with malonamidase, malonate and ammonia were produced by its hydrolytic activity. The rate of ammonia formation by the enzymes from *R. japonicum* and *R. meliloti* was determined by the method described above to be linear with respect to the reaction time and on the amount of enzyme. When hydroxylamine was included in the reaction mixture, malonohydroxamate was formed by its transmalonylation activity. Malonohydroxamate formation was identified by paper chromatography with a developing solvent, *n*-butanol-ethanol-water (2:2:1) (Fig. 5). Malonohydroxamate was also produced by the

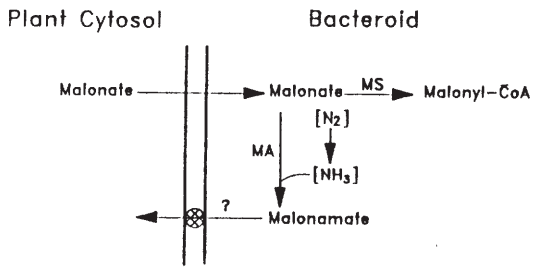

Fig. 6. *Proposed role of malonamidase(MA) and malonyl-CoA synthetase (MS) in Rhizobia.*

incubation of malonate with the enzymes from both sources in the presence of hydroxylamine. This malonohydroxamate was also identified by paper chromatography. Dicarboxylate dependent omega amidase was first reported by Meister *et al.* (Meister *et al.*, 1955). Evidence of the operation of omega amidase and glutamine transaminase as the enzymes responsible for the conversion of glutamine to alpha amino nitrogen, ammonium, and carbon skeletons was also reported in mammalian (Cooper and Meister, 1981) and microbial systems (Calderon, 1985; Soberon and Gonzalez, 1987). But malonate-specific omega amidase, malonamidase, has never been reported.

Kinetic constants

Increasing concentration of malonamate or malonate in the presence of hydroxylamine increased the rate of malonohydroxamate formaiton by the enzymes from *R. japonicum* and *R. meliloti*, and typical Michaelis-Menten substrate saturation patterns were obtained. From linear double reciprocal plots, K_ms were calculated as shwon in Table 3. K_ms (1.62 and 2.12 mM) of malonamate obtained by the two enzymes were ten times lower than that (16.2 and 22.0 mM) of malonate without source difference.

pH optimum

The optimum pH values for malonamidases from *R. japonicum* and from *R. meliloti* were 6.5 and 6.8, respectively.

In summary, two novel, malonate-specific enzymes were found in free living *Rhizobia*. *R. japonicum* contained both malonyl-CoA synthetase and malonamidase. However, *R. meliolti* contained only malonamidase, whereas *R. trifolii* had only malonyl-CoA synthetase. The substrate specificities of the two enzymes were highly specific for malonate. Low kinetic constants (K_m) indirectly indicate a physiological significance. Very recently it has been suggested

that malonamidases may play an important role for nitrogen flow in soybean nodule (Kim and Chae, 1990). At present the function of the enzymes are uncertain but malonamidase in *Rhizobia* may generally play a role as a carrier of ammonia generated by nitrogenase in symbiosis through the formation of malonamate (Fig. 6). Many enzymes capable of metabolizing reduced nitrogen have been detected in nodules (Robertson and Farnden, 1980), but it is difficult to determine the importance of each enzyme because many alternate metabolic pathways exist. Free living nitrogen-fixing bacteria are known to reduce nitrogen by inducing nitrogen assimilatory enzymes that can release ammonia from poor nitrogen sources, and to have a high-affinity transport system for nitrogen containing compounds in addition to enzymes related to nitrogen fixation (Roberts and Brill, 1981). *Rhizobium* bacteroids do not have these characteristics. In effective bacteroids, glutamine synthetase and glutamate synthase activities are low(Werner *et al.*, 1980) and the high affinity ammonia transport system is not present (O'Hara *et al.*, 1985). These results suggest that there may be nitrogen assimilatory enzymes other than glutamine synthetase and glutamate synthase and that there may also be a different transport system for fixed nitrogen other than the direct ammonia transport system. We propose that malonamate may be an important intermediate for nitrogen transport.

적 요

Malonate의 농도가 높은 식물의 뿌리에서 공생하는 *Rhizobium japonicum, Rhizobium meliloti*와 *Rhizobium trifolii*에서 malonate-specific한 두가지 효소, malonyl-CoA synthetase와 malonamidase를 발견하였다. Malonyl-CoA synthetase는 ATP/Mg^{2+} 존재하에서 malonate와 CoA로부터 직접 malonyl-CoA와 AMP, PPi를 합성한다. Malonamidase는 malonamate를 가수분해 또는 malonyl transfer반응을 하며 또 malonate와 hydroxylamine으로부터 직접 malonohydroxamate를 합성하는 새로운 효소이다. 두 효소들은 malonate에 대하여 높은 기질특이성을 나타낸다. 이러한 결과들은 *Rhizobia*에 malonate를 대사할 수 있는 효소들이 있다는 최초의 증거이며 malonate는 공생 과정에서 탄소 및 질소 대사에 중요한 역할을 할것이라는 것을 제시한다.

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