

Kinetics of Malonyl-CoA Synthetase from *Rhizobium trifolii* and Evidences for Malonyl-AMP Formation as a Reaction Intermediate

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The catalytic mechanism of malonyl-CoA synthetase from *Rhizobium trifolii* was investigated by the steady state kinetics and intermediate identification. Initial velocity studies and the product inhibition studies with AMP and PPi strongly suggested ordered Bi Uni Uni Bi Ping-Pong Ter Ter system as the most probable steady state kinetic mechanism of malonyl-CoA synthetase. Michaelis constants were $0.17 \pm 0.04 \mu\text{M}$, $0.24 \pm 0.18 \mu\text{M}$ and $0.045 \pm 0.026 \mu\text{M}$ for ATP, malonate and CoA, respectively. The TLC analysis of the ³²P-labelled products in reaction mixture containing [γ -³²P]ATP in the absence of CoA showed that PPi was produced after the sequential addition of ATP and malonate. Formation of malonyl-AMP, suggested as an intermediate in the kinetically deduced mechanism, was confirmed by the analysis of ³¹P-NMR spectra of AMP product isolated from the ¹⁸O transfer experiment using [¹⁸O]malonate. Two resonances were observed, corresponding to AMP labelled with zero and one atom of ¹⁸O, indicating that one atom of ¹⁸O transferred from [¹⁸O]malonate to AMP through the formation of malonyl-AMP. Formation of malonyl-AMP was also confirmed through the TLC analysis of reaction mixture containing [α -³²P]ATP. These results strongly support the ordered Bi Uni Uni Bi Ping-Pong Ter Ter mechanism deduced from the initial velocity and product inhibition studies.

Introduction

Malonyl-CoA synthetase, which catalyzes the formation of malonyl-CoA directly from malonate and CoA with the hydrolysis of ATP into AMP and PPi, has been purified and characterized from *Rhizobium trifolii*¹. This enzyme is 63,000 Da monomeric and it shows a high substrate specificity for malonate, ATP, and CoA. Pyridoxal-5'-phosphate, 2,3-butanedione, diethylpyrocarbonate, and N-bromosuccinimide severely inhibit the enzyme, indicating that essential amine, guanidino, imidazole, and indole groups are involved in catalysis²⁻⁴.

Malonyl-CoA synthetase and malonamidases as malonate-specific enzymes were discovered in Rhizobial species^{1,5,6}. *Bradyrhizobium japonicum* contain all the malonate-specific enzymes and *R. meliloti* contain only one type of malonamidase, whereas *R. trifolii* contain only malonyl-CoA synthetase. Therefore, it is important to define the enzymatic properties and the physiological role of *R. trifolii* malonyl-CoA synthetase. Recently malonyl-CoA synthetase has been proposed to play a role for the nitrogen flow in symbiosis¹.

Central to the understanding of any enzyme is the knowledge of the kinetic mechanism. Since malonyl-CoA synthetase catalyzes a reaction involving three substrates and three products (Ter Ter reaction), many different catalytic routes can be considered. We report here the results of a kinetic investigation of malonyl-CoA synthetase from *R. trifolii* and the evidences for the formation of malonyl-AMP: enzyme complex during the catalysis.

Experimentals

Materials. ATP, Sodium malonate, CoA, AMP, Dowex 1-X8, Inorganic pyrophosphatase (39 units/ml), polyethyleneimine (PEI)-cellulose plates, Hepes were purchased from

Sigma Chem. Co.: H₂¹⁸O (97 atom% enriched) from Aldrich Chem. Co.: [α -³²P]ATP (-3000 Ci/mmol) and [γ -³²P]ATP (-3000 Ci/mmol) from Amersham International.

Preparation of malonyl-CoA synthetase. Malonyl-CoA synthetase from *Rhizobium trifolii* ATCC 14479 grown on glucose as a carbon source was prepared by the method reported previously¹. Analysis of the purified enzyme by sodium dodesyl sulfate-polyacrylamide gel electrophoresis revealed that the enzyme was essentially homogeneous.

Malonyl-CoA synthetase assay. There are four different assay methods for the determination of malonyl-CoA synthetase activity⁷. Among them the malonohydroxamate assay was used for the preparation of the enzyme and ¹⁸O transfer experiment, while the direct spectrophotometric assay, based on the measurement of the increase in absorbance at 232 nm resulting from the formation of a thioester bond of malonyl-CoA ($\epsilon_{232} = 4.5 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$), was routinely used for kinetic experiments. The standard reaction mixture for direct spectrophotometric assay contained the following components in a final volume of 0.5 ml; 100 mM potassium phosphate buffer, pH 7.1; 2 mM sodium malonate; 5 mM MgCl₂; 0.1 mM ATP; 0.1 mM CoA, and the enzyme. Reactions were initiated with malonyl-CoA synthetase, and initial rates were obtained from the linear portion of the progress curve. All kinetic measurements were made in cuvettes of 1 cm path length with a Shimadzu UV-260 recording spectrometer equipped with a thermo-stated cell compartment. Constant temperature (30°C) was maintained in the cell compartment using a circulating water system.

Initial velocity experiments. Because three substrates are involved in the malonyl-CoA synthetase reaction, initial velocity experiments were carried out for the overall reaction in which the concentration of one substrate was varied in the presence of different levels of a second substrate, while the concentration of the third substrate was held

constant at non-saturating concentrations⁸. Under all conditions, the enzyme concentration ensuring linear relationships between incubation time and rates of reaction was chosen. We observed substrate inhibition over 0.6 mM for malonate, 0.3 mM for ATP, and 0.06 mM for CoA. However, the working concentrations of substrates were below their inhibition concentrations. The data reported here were all carried out in duplicate. The concentration of MgCl₂ was 5 mM throughout. Because the ATP concentrations utilized in the experiments never exceeded 0.3 mM, we have made the simplifying assumption that this concentration of MgCl₂ is sufficiently high that kinetic effects of Mg²⁺ need not be considered.

Kinetic data analysis. The kinetic data were analyzed graphically by constructing double reciprocal plots of initial velocities and substrate concentrations, followed by replots of slopes and intercepts against the reciprocal concentrations of changing fixed substrate in the initial velocity studies, or against the inhibitor concentration in the product inhibition studies; All plots and their replots were linear. Kinetic data were fitted to the appropriate initial velocity equation with the FORTRAN programs of Cleland to obtain the desired kinetic parameters⁹. Data from the initial velocity measurements were fitted to Eqs. (1) and (2).

$$v = VAB / (K_a K_b + K_a B + K_b A + AB) \quad (1)$$

$$v = VAB / (K_a B + K_b A + AB) \quad (2)$$

Where V is the maximum velocity; A and B are the concentrations of substrates A and B ; K_a , K_b are K_m constants for the substrates A and B , respectively; K_a is dissociation constant for the reaction of A with free enzyme. Because our measurements were made at a single constant value of the fixed substrate, the reported kinetic parameters are apparent values rather than true values.

Preparation of [¹⁸O] malonic acid. [¹⁸O] Malonate was synthesized by the reaction of malonyl dichloride with H₂¹⁸O. H₂¹⁸O (0.5 g) and malonyl dichloride (1.3 ml) were mixed in a round bottom flask (molar ratio 2 : 1). After one minute, malonic acid remained as a solid product. It was dissolved in 20 ml ethylacetate and decolorized with Norit. 20 ml benzene was added and the mixture was kept at -20 °C for 12 h. [¹⁸O] Malonic acid was crystallized and dried under reduced pressure, [¹⁸O] Malonic acid was methylated with ethereal diazomethane and analyzed by GC/MS to be [¹⁸O] malonic acid containing 50% [¹⁸O].

¹⁸O Transfer experiment. Reaction mixture contained 100 mM potassium phosphate buffer, pH 7.1, 5 mM MgCl₂, 4 mM [¹⁸O] malonate, 10 mM ATP, 0.5 mM CoA, 200 mM neutralized NH₂OH, and 0.06 units of malonyl-CoA synthetase in a total volume of 0.5 ml. Reaction was initiated with the enzyme and the mixture was incubated for 1 h at 37 °C. The reaction mixture was filtered, using a Centricon-30 ultrafiltration apparatus (Amicon Co.) to remove the protein, and the filtrate was loaded onto a Dowex 1-X8 column (3 × 2.5 cm). The adenine nucleotides were eluted with a 300 ml linear gradient of formic acid (0-5 M). AMP was eluted and its identity confirmed by TLC on PEI-cellulose plate with 0.7 M sodium phosphate buffer, pH 3.5, as a developing solvent. AMP fractions were pooled, lyophilized, and then dissolved in 10 ml of distilled water. The pH of the AMP solution was adjusted with diluted NaOH to about pH 8.5 and again

lyophilized. The lyophilized AMP was dissolved in 1.3 ml of 20 mM Hepes buffer, pH 8.8, containing 1 mM EDTA and 20% D₂O. The solution of commercial AMP (11 mM) was prepared in the same condition as above and was used as a standard. The ³¹P-NMR measurements of standard and sample AMP were performed as described below.

NMR measurements. The ³¹P-NMR spectra (121.47 MHz) were obtained in the Fourier-transform mode with a Varian Gemini-300 7.05T spectrometer. NMR measurements were made at 25 ± 1 °C with a sample volume of 0.8 ml contained in a 5 mm tube. A total of 246 transients were accumulated using pulses of 45° (the 90° pulse was 16 μs) and an acquisition time of 3.0 s. A sensitivity enhancement exponential function gave a line broadening of 0.1 Hz. The chemical shifts are reported relative to 85% H₃PO₄ as an external reference.

Results and Discussion

Initial velocity studies. Evaluation of the kinetic mechanism and, in particular, the kinetic parameters of an enzyme-catalyzed reaction can often be best approached through the initial velocity studies. Double-reciprocal plots of the initial velocity data allow the differentiation of the kinetic mechanism; ping-pong mechanisms result in one or more parallel lines in double-reciprocal plots, whereas sequential mechanisms exhibit non-parallel lines. The results of an experiment, in which malonate and ATP concentrations were varied at different fixed concentrations of CoA, are presented in double-reciprocal form in Figure 1A and 1B, respectively. In both cases, the lines are parallel. This behaviour is characteristic of the ping-pong mechanism in which one or two products are released before the introduction of a final substrate. It also indicates that CoA is the final substrate introduced¹⁰. When the initial velocity data of Figure 1A and 1B were plotted with malonate and ATP as the variable substrates, respectively, both sets of results again yielded families of parallel lines, suggesting that the malonate-CoA pair and ATP-CoA pair interaction in the malonyl-CoA synthetase-catalyzed reaction is a ping-pong mechanism. The data fitted well to Eq. (2) and the apparent values for the kinetic constants were summarized in Table 1. There are three classes of ping-pong Ter Ter mechanisms; Bi Uni Uni Bi ping-pong Ter Ter mechanism, Bi Bi Uni Uni ping-pong Ter Ter mechanism, and Hexa Uni ping-pong mechanism. The effects of ATP and malonate concentration changes on the initial rates (at constant non-saturating concentration of CoA) are depicted in Figure 1C. The lines are intersecting, indicating the sequential addition of ATP and malonate (or malonate and ATP) prior to product release. The data fitted well to Eq. (1) and the kinetic parameters are reported in Table 1. From these results, Hexa Uni ping-pong Ter Ter mechanism could be ruled out. In the same experiment as in Figure 1, but with the concentration of CoA, ATP, or malonate each raised 4-fold (a near-saturating concentration), again the same patterns of double-reciprocal plots emerge. Thus, the same patterns of the double-reciprocal plots at constant saturating and non-saturating concentration of each substrate (ATP, malonate, and CoA) suggest that there is no random sequence in malonyl-CoA synthetase-catalyzed reaction.

Taken together, the initial velocity data cited above can

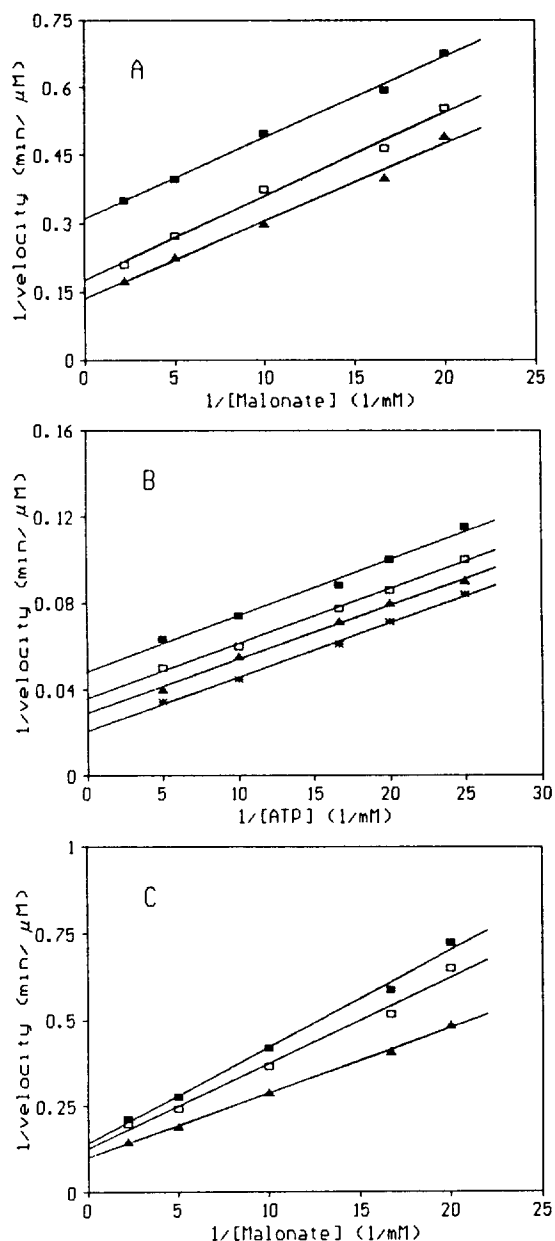


Figure 1. Initial velocity patterns on malonyl-CoA synthetase reaction. The reactions were initiated with 3×10^{-3} unit of purified malonyl-CoA synthetase. (A) Plots of $1/v$ versus $1/[\text{Malonate}]$ at various levels of CoA, and at a constant concentration of ATP (100 μM). CoA concentrations were 10 μM (■), 12.5 μM (□), 25 μM (▲). (B) Plots of $1/v$ versus $1/[\text{ATP}]$ at various levels of CoA, and at a constant concentration of malonate (0.2 μM). CoA concentrations were 8 μM (●), 12.5 μM (□), 16 μM (▲), 25 μM (*). (C) Double-reciprocal plots of the initial velocity versus malonate concentration at several concentrations of ATP, and a fixed concentration of CoA (25 μM). ATP concentrations were 50 μM (■), 60 μM (□), 100 μM (▲).

be fitted to the rate equation for Bi Uni Uni Bi or Bi Bi Uni Uni ping-pong Ter Ter mechanism. In the absence of product, the initial velocity equation for the two mechanism is the same (Eq. 3).

$$v = VABC / (K_{ia}K_bC + K_aAB + K_bAC + K_cBC + ABC) \quad (3)$$

Table 1. Kinetic Constants for Malonyl-CoA Synthetase

Kinetic constant	App value (mM)
$K_{i \text{ ATP}}$	0.11 ± 0.02^a
K_{ATP}	0.037 ± 0.009^a
	0.31 ± 0.04^b
K_{malonate}	0.1 ± 0.02^a
	0.38 ± 0.18^b
K_{CoA}	0.045 ± 0.025^b
	0.045 ± 0.006^b

^aValue obtained by fitting the data to Eq. (1). ^bValue obtained by fitting the data to Eq. (2).

Where V is the maximum velocity; A , B , and C are the concentrations of the three substrates; K_a , K_b , K_c are K_m constants for the substrates A , B , and C , respectively; K_{ia} is the dissociation constant for the reaction of A with free enzyme. The rate equation given in Eq. (3) may be rearranged according to which one of the substrates is fixed to yield Eqs. (4)-(6).

Varying B and C at a constant level of A :

$$v = VBC / (K_{ia}K_bC/A + K_aB + K_bC + K_aBC/A + BC) \quad (4)$$

Varying A and C at a constant level of B :

$$v = VAC / (K_{ia}K_bC/B + K_aA + K_aAC/B + K_cC + AC) \quad (5)$$

Varying A and B at a constant level of C :

$$v = VAC / (K_{ia}K_b + K_aAB/C + K_aA + K_aB + AB) \quad (6)$$

The initial velocity data obtained above were well fitted to these equations (Eqs. 4-6).

Product inhibition studies. Although the initial velocity data give a good deal of information for the kinetic mechanism of malonyl-CoA synthetase reaction, our initial velocity data should be supplemented with experiments involving product inhibitors in order to differentiate between Bi Uni Uni Bi and Bi Bi Uni Uni ping-pong Ter Ter mechanisms, and to determine the most likely pathway of substrate addition to the enzyme. The most commonly used method involves holding two substrates at non-saturating concentrations, while varying the third substrate and inhibitor concentrations. The initial velocity data are then plotted in double-reciprocal form, and the inhibition patterns can be used to limit the possible kinetic pathways.

AMP was found to act as an uncompetitive inhibitor of CoA in the malonyl CoA synthetase reaction (Figure 2A). Competitive inhibition was also observed with respect to ATP (Figure 2B). Taken along with the results from the initial velocity experiments, these inhibition patterns indicate that AMP is the R product (final released product) and that substrate A (the first substrate to bind) is ATP and substrate B is malonate¹⁰. Therefore, Bi Bi Uni Uni ping-pong Ter Ter mechanism is ruled out and Bi Uni Uni Bi mechanism is left as the most reasonable kinetic pathway in malonyl-CoA synthetase reaction. Bi Uni Uni Bi ping-pong Ter Ter mechanism for malonyl-CoA synthetase reaction was further supported by product inhibition with PPI. PPI behaved as a mixed-type inhibitor with respect to malonate (Figure 2C). In the presence of the product, new terms are present in the denominator of the velocity equation (Eq. 7-9).

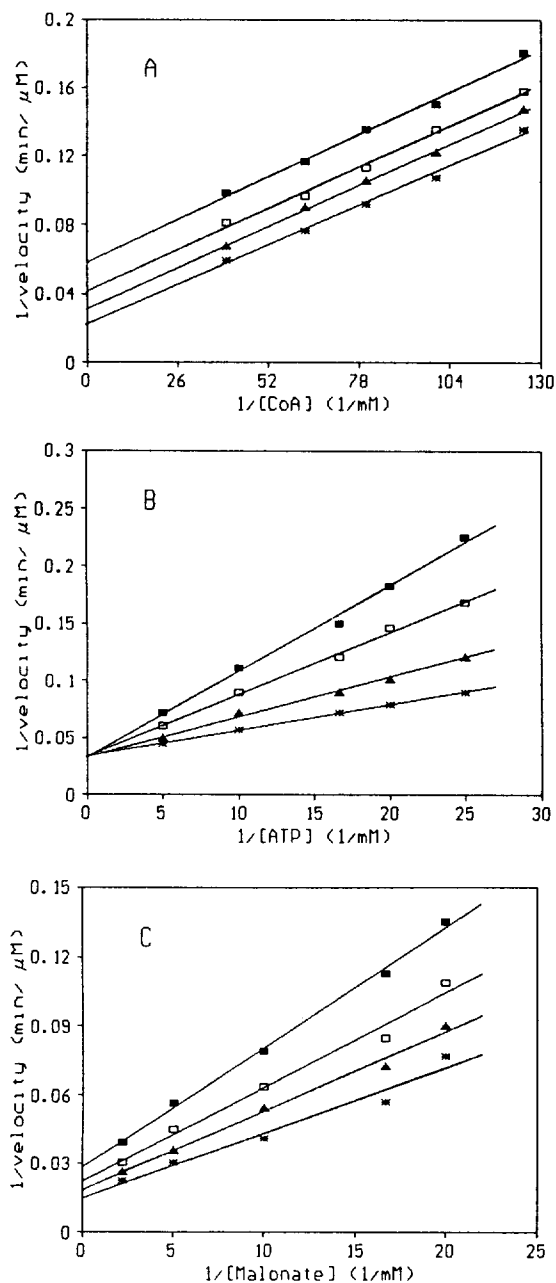


Figure 2. Product inhibition patterns on malonyl-CoA synthetase reaction. (A) Double-reciprocal plots of the initial velocity versus the CoA concentration at various concentrations of AMP. Malonate and ATP concentrations were fixed at 0.2 mM and 0.1 mM, respectively. AMP concentrations were 100 μM (■), 60 μM (□), and 40 μM (▲); * indicates the initial velocities in the absence of inhibitor. (B) Double-reciprocal plots of the initial velocity versus the ATP concentration at various concentrations of AMP. Malonate and CoA concentration at various concentrations of AMP. Malonate and CoA concentrations were fixed at 0.2 μM and 16 μM , respectively. AMP concentrations were 100 μM (■), 60 μM (□), and 40 μM (▲); * indicates the initial velocities in the absence of inhibitor. (C) Double-reciprocal plots of the initial velocity versus the malonate concentration at various concentrations of PPI. ATP and CoA concentrations were 0.1 mM and 16 μM , respectively. PPI concentrations were 100 μM (■), 60 μM (□), and 20 μM (▲); * is the initial velocity data obtained in the absence of inhibitor.

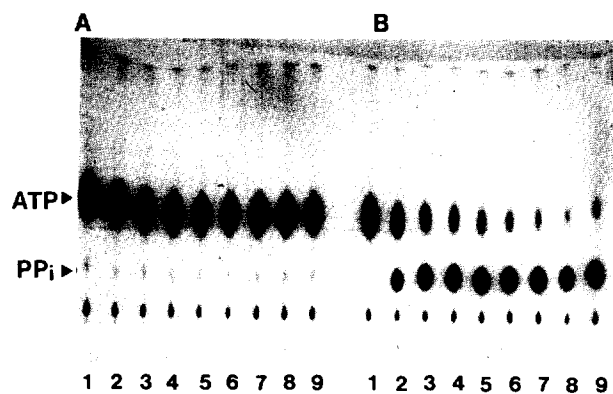


Figure 3. Autoradiogram of TLC analysis of the partial reaction products using $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. The basic reaction mixtures contained 0.1 M potassium phosphate (pH 7.1) buffer, 5 mM MgCl_2 , 0.2 pmol $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, and 5×10^{-3} unit of malonyl-CoA synthetase (4.1 pmol) in a total volume of 25 μl . Reactions were initiated with the addition of enzyme. The mixture was quickly mixed with a vortex mixer and incubated at 37°C. The aliquots of 1 μl were taken at the indicated time and spotted onto a PEI-cellulose plate. 0.7 M Sodium phosphate (pH 3.5) buffer was used as a developing solvent. (Panel A) without malonate. (Panel B) added malonate to a final concentration of 0.2 mM. Lane 1, control (with boiled enzyme); lane 2, 1 min; lane 3, 3 min; lane 4, 5 min; lane 5, 7 min; lane 6, 9 min; lane 7, 12 min; lane 8, 15 min; lane 9, 20 min. The position of ATP was identified using unlabeled ATP, while the position of Pi and PPI were deduced from the result of pyrophosphatase treatment.

When C is varied in the presence of R

$$v = VC / [K_c + C(1 + K_a/A + K_b/B + K_{ia}K_b/AB + K_{ia}K_bR/K_{ip}AB + K_cR/K_{ip}A)] \quad (7)$$

When A is varied in the presence of R

$$v = VA / [K_a(1 + K_{ia}K_b/K_aB + K_{ia}K_bR/K_aK_{ip}B) + A(1 + K_b/B + K_c/C)] \quad (8)$$

When B is varied in the presence of R

$$v = VB / [K_b(1 + K_{ia}/A)(1 + K_{ip}K_cP/K_bK_{ip}C) + B(+K_a/A + K_c/C + K_cP/K_{ip}C)] \quad (9)$$

The product inhibition data obtained above were well fitted to the appropriate rate equations (Eqs. 7-9). The apparent values for the inhibition constants obtained from the secondary replots were: AMP has a K_i value of $53.4 \pm 10.0 \mu\text{M}$ with respect to CoA and of $35.4 \pm 12.1 \mu\text{M}$ with respect to ATP; PPI has a K_i value of $125 \pm 11 \mu\text{M}$ with respect to malonate.

Identification of PPI as the first product. Since the kinetic analysis shown above suggested that PPI was the first product to be released, the formation of PPI was monitored using $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ in the partial reaction mixture containing no CoA. As shown in Figure 3, PPI was formed after the sequential addition of ATP and malonate. In order to determine the order of ATP and malonate introduced, the isolated of enzyme-ATP (or enzyme-malonate) complex from the partial reaction mixture containing only ATP (or malonate) as a substrate was attempted by gel filtration. But the attempts failed, indicating that ATP or malonate alone may not form a stable complex with the enzyme.

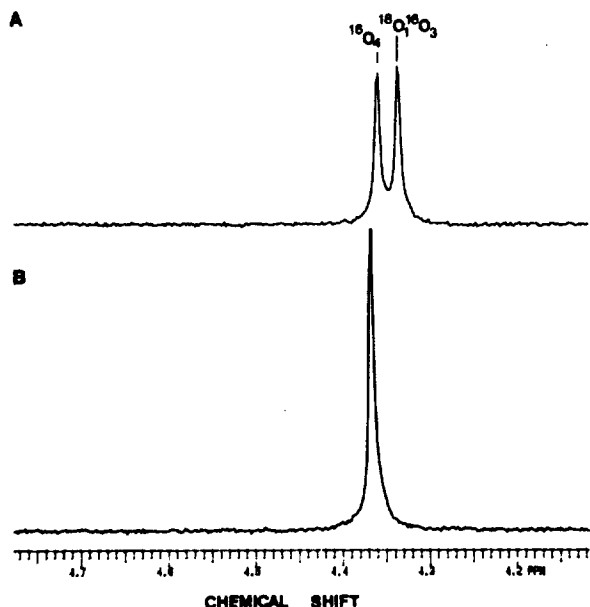


Figure 4. ³¹P-NMR spectrum of the AMP product of the ¹⁸O transfer experiment. (Panel A) AMP product. The relative integrals of the peaks are 0.96 and 1.00 (Panel B) Standard AMP.

The evidences for the formation of malonyl-AMP.

From the initial velocity and the product inhibition studies, the formation of malonyl-AMP, an intermediate in the Bi Uni Uni Bi ping-pong mechanism, was suggested. The formation of the intermediate was confirmed by ¹⁸O transfer from [¹⁸O] malonate to AMP. ¹⁸O-labeled malonate (50% ¹⁸O) was supplied as a substrate together with ATP and CoA in the malonyl-CoA synthetase-catalyzed reaction. The reaction was carried out to 99% completion by the elimination of malonyl-CoA as malonohydroxamate in the presence of NH₂OH. AMP, one of the products, was isolated from the reaction mixture by using Dowex 1-X8 anion exchanger. The ¹⁸O content in the AMP was monitored by ³¹P-NMR. As shown in Figure 4A, two resonances were observed corresponding to AMP labelled with zero and one atom of ¹⁸O. When unlabelled AMP (used as standard) was added to the sample, the down-field resonance increased in intensity, indicating that this peak was indeed unlabelled AMP (data not shown). The separation between the two resonances was 0.024 ppm, consistent with the chemical shift per ¹⁸O reported by Cohn and Hu¹¹. The resonance integrals were almost identical (Figure 4A), indicating that 50% of AMP product was labelled with one atom of ¹⁸O. These results clearly show that malonyl-AMP was formed during the malonyl-CoA synthetase reaction.

The formation of malonyl-AMP was also confirmed by TLC analysis of a reaction mixture containing the enzyme, malonate, and [^{α-³²P}]ATP, which revealed the presence of a novel nucleotide derivative that was neither AMP, ADP, or ATP. Since the malonyl-AMP: enzyme complex may be very unstable in the presence of Mg²⁺, such as in the case of aminoacyl-tRNA synthetase¹²⁻¹⁴, we expected that the intensity of the AMP spot would increase with time. However, as shown in Figure 5A, the intensity in the AMP position showed only a temperature increase, concomitant with the appearance of

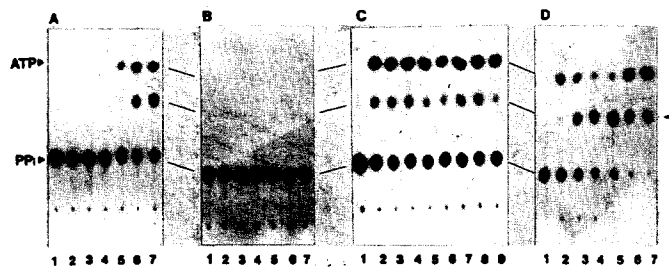


Figure 5. Autoradiogram of TLC analysis of reaction products using [^{α-³²P}]ATP. The basic reaction mixtures contained 0.1 M potassium phosphate (pH 7.1) buffer, 5 μM MgCl₂, 0.2 pmol [^{α-³²P}]ATP, other components in the indicated amount, and 7.5 × 10⁻³ unit of malonyl-CoA synthetase (6 pmol) in a total volume of 25 μl. All other conditions are as described in the legend of Figure 3. (Panel A) Lane 1, control (with boiled enzyme); lane 2-4, 1 min, 4 min, and 8 min in reaction with only [^{α-³²P}]ATP as a substrate, respectively; lane 5-7, 1 min, 4 min, and 8 min after subsequent addition of malonate to final concentration of 2 mM, respectively. (Panel B) The explanation for each lanes are the same as described in panel A except for the addition of 2 mM succinate in place of malonate. (Panel C) The basic reaction mixtures were first incubated for 8 min at 37°C. Malonate (final concentration, 2 μM) and inhibitor (in the indicated concentration) mixed previously were added and the mixtures were quickly mixed with a vortex mixer. The incubation was continued for 8 min at 37°C. Lane 1, control (with boiled enzyme); lane 2, only malonate without inhibitor; lane 3-6, 0.1 μM, 1 μM, 10 μM, and 100 μM AMP respectively; lane 7-9, 0.2 mM, 2 mM, and 20 mM succinate respectively. (Panel D) The basic reaction mixtures contained [^{α-³²P}]ATP and malonate (final concentration of 1 mM) as substrates. After 4 min, aliquots of 10 ml were taken and mixed with CoA (final concentration of 0.5 mM). The mixture containing CoA was separately incubated and aliquots of 1 μl were spotted onto a PEI-cellulose plate at the indicated time. Lane 1, control (with boiled enzyme); lane 2-5, 1 min, 4 min, 8 min, and 12 min from the reaction mixture in the absence of CoA, respectively; lane 6-7, 4 min and 8 min after the addition of CoA, respectively. The positions of each nucleotides were identified using unlabelled ATP, ADP, and AMP. The arrow indicates the novel nucleotide derivative, defined as malonyl-AMP.

a new spot. The intensity of the new spot, corresponding to a novel nucleotide derivative, gradually increased with the addition of malonate. However, this spot did not appear with the addition of succinate in place of malonate, indicating that the novel nucleotide derivative was formed from malonate and [^{α-³²P}]ATP (Figure 5B). The transient formation of AMP in the presence of malonate can be explained by the formation of a relatively stable transition-state trigonal bipyramid complex of malonate and ATP. The formation of the novel nucleotide derivative was inhibited by AMP and succinate (Figure 5C). Also, the novel nucleotide derivative disappeared gradually with the addition of CoA, concomitant with the production of AMP (Figure 5D). Combined results of the experiments using [^{α-³²P}]ATP clearly indicate that the novel nucleotide derivative is malonyl-AMP.

Proposed mechanism of malonyl-CoA synthetase catalysis. The results from initial velocity and product

