Purification and Properties of Malyl-Coenzyme A Lyase from *Pseudomonas* AM1

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1. Malyl-CoA lyase was purified 20-fold from extracts of methanol-grown *Pseudomonas* AM1. 2. Preparations of the enzyme were essentially homogeneous by electrophoretic and ultracentrifugal criteria. 3. Malyl-CoA lyase has a molecular weight of 190 000 determined from sedimentation-equilibrium data. 4. Within the range of compounds tested, malyl-CoA lyase is specific for (2*S*)-4-malyl-CoA or glyoxylate and acetyl-CoA or propionyl-CoA. 5. A bivalent cation is essential for activity, Mg^{2+} or Co^{2+} being most effective. 6. Malyl-CoA lyase is inhibited by (2*R*)-4-malyl-CoA and by some buffers, but thiol-group inhibitors are without effect. 7. Optimal activity was recorded at pH 7.8. 8. An equilibrium constant of 4.7×10^{-4} M was determined for the malyl-CoA cleavage reaction. 9. The Michaelis constants for the enzyme are: 4-malyl-CoA, 6.6×10^{-5} M; acetyl-CoA, 1.5×10^{-5} M; glyoxylate, 1.7×10^{-3} M; Mg^{2+} , 1.2×10^{-3} M.

It has been shown (Salem *et al.*, 1973) that extracts of methanol-grown *Pseudomonas* AM1 contain in high activity a malyl-CoA lyase catalysing the reversible reaction:

This reaction, working in the direction of malyl-CoA cleavage, may be of key importance in regenerating the glyoxylate necessary for operation of the serine pathway during growth of *Pseudomonas* AM1 on C_1 compounds.

The present paper records a study of the purification and properties of malyl-CoA lyase obtained from methanol-grown *Pseudomonas* AM1.

Materials and Methods

Chemicals

(*S*)-(β-Hydroxysuccinyl)-*N*-octanoylcysteamine [(*S*)-(β-hydroxy-3-carboxypropionyl)-*N*-octanoylcysteamine] was prepared from *S*-malate, octanoic acid and cysteamine by the methods described by Eggerer & Grünwälder (1964) and it was used to make (2*S*)-4-malyl-CoA by ester interchange with CoA as previously described (Salem *et al.*, 1973). (2*R*)-4-Malyl-CoA was similarly prepared from (*R*)-(β-hydroxysuccinyl)-*N*-octanoylcysteamine. *S*-Citryl-CoA was prepared from *S*-citryl-*N*-octanoylcysteamine by the method of Eggerer (1963). *S*-Citryl-*N*-octanoylcysteamine and (*R*)-(β-hydroxysuccinyl)-*N*-octanoylcysteamine were gifts from Professor H. Eggerer (Universität Regensburg, Germany).

Acetyl-CoA and succinyl-CoA were prepared by reaction of their respective acid anhydrides with CoA and (DL)-3-hydroxybutyryl-CoA was prepared by reaction of the mixed anhydride of ethyl hydrogen

carbonate and (DL)-3-hydroxybutyric acid with CoA. Both procedures were as described by Stadtman (1957). Oxalyl-CoA was prepared by ester interchange between thiocresyl-oxalic acid and CoA as described by Quayle (1962, 1963). Propionyl-CoA, *n*-butyryl-CoA and 3-hydroxymethylglutaryl-CoA were purchased from P-L Biochemicals Inc., Milwaukee, Wis., U.S.A. Succinyl-CoA, 3-hydroxybutyryl-CoA, 4-malyl-CoA and oxalyl-CoA were assayed chemically from the decrease in absorbance at 232 nm (succinyl-CoA and 3-hydroxybutyryl-CoA), 234 nm (4-malyl-CoA) or 270 nm (oxalyl-CoA), associated with their hydrolysis in 0.1 M-NaOH at 30°C for 10 min. Citryl-CoA was incubated in 0.1 M-NaOH at 30°C until no further decrease in E_{235} could be detected (approx. 8 h). The differences in molar extinction coefficients of the acyl-CoA derivatives and their products were taken to be 4.5×10^3 litre·mol⁻¹·cm⁻¹ for succinyl-CoA, 3.7×10^3 litre·mol⁻¹·cm⁻¹ for 3-hydroxybutyryl-CoA, 4.8×10^3 litre·mol⁻¹·cm⁻¹ for 4-malyl-CoA, 2.3×10^3 litre·mol⁻¹·cm⁻¹ for oxalyl-CoA and 4.7×10^3 litre·mol⁻¹·cm⁻¹ for citryl-CoA.

Acetyl-CoA was assayed enzymically by arsenolysis in the presence of phosphate acetyltransferase (acetyl-CoA orthophosphate acetyltransferase, EC 2.3.1.8) and arsenate (Stadtman, 1957) or conversion into citrate and CoA in the presence of oxaloacetate and citrate synthase [citrate oxaloacetate-lyase (CoA-acetylating), EC 4.1.3.7] followed by assay of CoA with 5,5'-dithiobis-(2-nitrobenzoate) (Tubbs & Garland, 1969).

Calcium phosphate gel was prepared by the method described by Keilin & Hartree (1938).

DEAE-cellulose (pre-swollen DE-52) was obtained from Whatman Biochemicals Ltd., Springfield Mill, Maidstone, Kent, U.K.

Purified enzymes, nucleotides and CoA were purchased from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K.

Ultracentrifugation

A Beckman model E analytical ultracentrifuge with an AN-D type rotor was used throughout. Before centrifugation the enzyme preparations were dialysed against 1000 vol. of 0.15 M-KCl-0.05 M-KH₂PO₄ (pH 7.0) for 24-48 h at 0-4°C. During sedimentation-velocity analysis, enzyme at a concentration of 4.8 mg/ml was examined in a single-sector cell with a Dural centrepiece by using a schlieren optical system. Photographs were taken every 8 min on Ilford Selochrome plates. Sedimentation-equilibrium analysis by the high-speed method of Yphantis (1964) was performed by using a Rayleigh interference optical system and a double-sector cell with an Epon centrepiece and sapphire windows. Enzyme solution (100 μ l; E_{280} 0.65-0.9) was layered on to FC-43 fluorocarbon oil (10 μ l) in one sector of the cell and an equal volume of buffer was layered on to oil in the other sector. The centrifuge was operated at 15 220 rev./min and photographs were taken on Ilford G-72 formolith-backed plates after 64 h by using a 4 min exposure.

Electrophoresis of protein

Polyacrylamide-disc-gel electrophoresis was performed at pH 8.5 as described by Davis (1964). As a routine, gels at a concentration of 7.5% (w/v) acrylamide were used, but to examine the homogeneity of purified preparations 5% and 10% gels were used in addition. Amido Black [0.1% in 7% (v/v) acetic acid] was used as a staining agent.

Enzyme assays

Spectrophotometric results were obtained with a Unicam SP.1800 recording spectrophotometer. All enzyme assays were conducted at 30°C.

Malate synthase [L-malate glyoxylate-lyase (CoA-acetylating), EC 4.1.3.2]. This enzyme was assayed by the method of Dixon & Kornberg (1962).

Citrate synthase. This enzyme was assayed by the method of Srere (1969).

Malyl-CoA lyase. This was assayed as a routine in the direction of cleavage to acetyl-CoA and glyoxylate with added phenylhydrazine by the continuous method described by Salem *et al.* (1973). For measurement of the pH optimum of the reaction, the glyoxylate produced was determined discontinuously, as described in the text. The enzyme was also assayed discontinuously in the direction of 4-malyl-CoA synthesis by measuring the disappearance of acetyl-CoA or glyoxylate. Reaction

mixtures contained, in a 2.0 ml final volume, potassium phosphate buffer, pH 7.4 (200 μ mol), MgCl₂ (20 μ mol), acetyl-CoA (1 μ mol) and potassium glyoxylate (1 μ mol). The reaction was started by the addition of enzyme extract (1-5 μ g of protein). At convenient time-intervals, samples (250 μ l) were withdrawn and 50 μ l of 3.0 M-HClO₄ was added to each. After incubation for 10 min at room temperature the samples were adjusted to neutral pH by the addition of 2.5 M-K₂CO₃ and the precipitate of KClO₄ was removed by centrifugation. A sample (200 μ l) of the supernatant was then added to a cuvette containing (in 800 μ l) potassium phosphate buffer, pH 7.4 (100 μ mol), and phenylhydrazine (5 μ mol). The E_{324} was measured against a similar reaction mixture to which 200 μ l of water had been added in place of the sample. In some instances, 4-malyl-CoA concentration was also measured at this stage by adding purified malyl-CoA lyase and MgCl₂ (10 μ mol) and recording the subsequent increase in E_{324} . Alternatively, malyl-CoA concentration was measured in the supernatant after precipitation of KClO₄ by hydrolysis with citrate synthase (Eggerer *et al.*, 1964). Reaction mixtures contained, in 1 ml, Tris-HCl buffer, pH 8.0 (100 μ mol), 5,5'-dithiobis-(2-nitrobenzoate) (0.1 μ mol) and sample (200 μ l). The increase in E_{412} after the addition of citrate synthase (100 μ g) was measured. Acetyl-CoA in the same sample was then determined by measuring the subsequent increase in E_{412} on the addition of oxaloacetate (0.1 μ mol).

Maintenance and growth of the organism

Pseudomonas AM1 (N.C.I.B. 9133) was maintained in slope culture on an inorganic-salts medium (Jayasuriya, 1955) containing methylamine hydrochloride (50 mm) and agar (1.5%, w/v). The organism was subcultured monthly and stored at 4°C after incubation for 3-4 days at 30°C. Liquid cultures were prepared as described by Heptinstall & Quayle (1970) with methanol (0.5%, v/v) as carbon source.

Purification of malyl-CoA lyase

Step 1: preparation of cell-free extract. Methanol-grown bacteria (60 g wet wt.) were suspended in 300 ml (final volume) of 0.1 M-potassium phosphate buffer, pH 7.0, and were circulated twice through a Rapi-dis F400 continuous sonicator (Ultrasonics Ltd.) at a flow rate of 8 ml/min with power and tuning both at setting 7. The suspension was cooled on ice and circulated at 0-10°C. The resulting extract was centrifuged at 30 000g for 40 min at 4°C and the pellet discarded.

Step 2: protamine sulphate treatment. To the supernatant was added protamine sulphate (15 mg/ml

in 0.1 M-potassium phosphate buffer, pH 7.0) dropwise and with continuous stirring to a final concentration of 1 mg/10 mg of protein. The solution was equilibrated for 30 min at room temperature, and the resultant precipitate was removed by centrifugation (30000g for 20 min) and discarded.

Step 3: $(\text{NH}_4)_2\text{SO}_4$ precipitation. To the supernatant was added, with stirring, $(\text{NH}_4)_2\text{SO}_4$ to 45% saturation at 4°C. The pH of the solution was maintained at 7.0 by addition of 2 M- NH_3 . After equilibration at 4°C for 30 min the resultant precipitate was removed by centrifugation and discarded. To the supernatant was added $(\text{NH}_4)_2\text{SO}_4$ to 55% saturation. After equilibration as above the precipitate was collected by centrifugation and the supernatant discarded. The precipitate was dissolved in 50 ml of 0.1 M-potassium phosphate buffer, pH 7.0, and dialysed overnight against 2 litres of 0.01 M-potassium phosphate buffer containing 0.01 M- MgCl_2 , pH 7.0.

Step 4: adsorption on calcium phosphate gel. To the dialysed $(\text{NH}_4)_2\text{SO}_4$ fraction was added calcium phosphate gel in a ratio 1:1 (w/w) to protein. The suspension was equilibrated at 4°C with stirring for 30 min. The gel was removed by low-speed centrifugation (2000g for 10 min) and discarded. A second addition of gel was made in a 5:1 (w/w) ratio to protein and after equilibration and centrifugation the supernatant was discarded. The gel was resuspended in 40 ml batches of 0.1 M-potassium phosphate buffer, pH 7.0, and after equilibration and centrifugation the supernatants were assayed for maly-CoA lyase activity. Normally two extractions were sufficient to elute 80–90% of the enzyme activity from the gel.

Step 5: DEAE-cellulose chromatography. The combined eluates from calcium phosphate gel were diluted with water until they were 0.05 M with respect to phosphate buffer (pH 7.0) and they were then applied to a DEAE-cellulose column (6.0 cm $\times$ 1.2 cm) which had been equilibrated with this buffer. The

column was washed with 50 ml of 0.05 M-potassium phosphate buffer and protein was thereafter eluted by a linear gradient of 0.05–0.15 M-potassium phosphate buffer, pH 7.0 (600 ml total). Fractions (7 ml) were collected and maly-CoA lyase activity was eluted at a concentration of approx. 0.1 M-phosphate in fractions 32–51. The purification procedure is summarized in Table 1.

Concentration of protein samples

Dilute fractions of DEAE-cellulose-column effluent containing maly-CoA lyase activity were concentrated by filtration under pressure [N_2 gas at 10^5 Pa (1 kg/cm²)] through PM30 membranes (Diaflo ultrafiltration membranes; Amicon Corp., Lexington, Mass., U.S.A.). Where protein solutions were required at concentrations of approx. 5 mg/ml for sedimentation-velocity analysis, protein was applied to a small (2.5 cm $\times$ 0.6 cm) column of DEAE-cellulose equilibrated with 0.05 M-potassium phosphate buffer, pH 7.0, and was eluted with 0.15 M buffer. The recovery of enzyme activity was 90–95%.

Protein determinations

Protein was measured by the Folin-Ciocalteu method as described by Lowry *et al.* (1951) with bovine serum albumin as standard.

Results

The properties of the maly-CoA lyase, purified as described in the Materials and Methods section, were studied as follows.

Criteria of purity

Ultracentrifugation. The preparation of purified maly-CoA lyase sedimented as a symmetrical peak, although traces of a lower-molecular-weight impurity

Table 1. Purification of maly-CoA lyase from *Pseudomonas* AM1

Experimental details are given in the Materials and Methods section. A unit of enzyme activity is defined as that quantity which will catalyse the cleavage of 1 μmol of substrate/min at 30°C. Values in parentheses refer to peak fraction from DEAE-cellulose.

Step	Enzyme activity (units/ml)	Volume (ml)	Total activity (units)	Recovery (%)	Protein (mg/ml)	Specific activity (units/mg)	Purification
Cell-free extract	15.8	255	4029	100	11.5	1.37	1
Protamine sulphate precipitation	13.6	280	3819	94.8	8.6	1.58	1.15
45–55%-satd. $(\text{NH}_4)_2\text{SO}_4$ fraction (after dialysis)	37.9	60	2276	56.5	7.3	5.2	3.8
Calcium phosphate gel	13.9	80	1112	27.6	0.95	14.6	10.7
DEAE-cellulose chromatography	2.8 (5.34)	140	392	9.7	0.105 (0.19)	26.6 (28.1)	19.4 (20.5)

Fig. 1. *Sedimentation-velocity pattern of malyl-CoA lyase*
The photograph was taken 49 min after a speed of 54740 rev./min was reached at 20°C. The protein concentration was 4.8 mg/ml in 0.15 M-KCl-0.05 M-KH₂PO₄, pH 7.0.

were possibly present (Fig. 1). A sedimentation coefficient of 9.8S was determined under the conditions described. The sedimentation-equilibrium data also gave results consistent with a homogeneous preparation; plots of log (fringe displacement) versus the square of the distance from the centre of rotation were linear.

Electrophoresis. When the preparation was subjected to disc electrophoresis on 7.5% (w/v) polyacrylamide gels at pH 8.5 a single protein band was observed. Changes in the pore size of the gel failed to resolve the preparation into more than one component. To obtain confirmation of the identity of the band, gels run at 0°C were sectioned and the segments homogenized in 0.1 M-potassium phosphate buffer-0.01 M-MgCl₂, pH 7.4. After equilibration for 15 min, the supernatants were assayed for malyl-CoA lyase activity. This could only be detected in segments corresponding to the stained bands.

Molecular weight

The molecular weight of malyl-CoA lyase was estimated by using the meniscus-depletion method of Yphantis (1964). By assuming a partial specific volume of 0.73 for the protein, a value of $190\,000 \pm 4000$ for the molecular weight was determined.

Stability

Purified enzyme preparations retained over 95% of their initial activity during storage periods of up to several months at -15°C. The enzyme was also stable when stored at 0-4°C for 2-3 weeks, but activity was rapidly and irreversibly lost at temperatures above 55°C.

Specificity

Purified malyl-CoA lyase preparations possessed no detectable malate synthase or citrate synthase activities when assayed by the standard procedures for these enzymes.

Substitution of (2*S*)-4-malyl-CoA by an equimolar amount of the optical isomer (2*R*)-4-malyl-CoA in the cleavage assay system resulted in a slight initial rise in E_{324} , but this stopped after production of glyoxylate corresponding to only 10% of the substrate supplied (as estimated by alkaline hydrolysis of the thioester bond). Contamination of the preparation of (2*R*)-4-malyl-CoA by 2-*S* isomer was investigated by using citrate synthase (Eggerer *et al.*, 1964) and by hydrolysis of the thioester bond under mild conditions (0.1 M-NaOH at 25°C) followed by determination of 2*S*-malate in the presence of NAD, acetyl-CoA, malate dehydrogenase and citrate synthase (Cornforth *et al.*, 1970). Both methods indicated that the sample of (2*R*)-4-malyl-CoA was stereochemically impure. It seems most likely that the enzyme was inactive towards (2*R*)-4-malyl-CoA and that the reaction observed was due to the presence of 2-*S* form in the substrate.

Citryl-CoA was tested as a possible substrate of malyl-CoA lyase with respect either to cleavage to oxaloacetate and acetyl-CoA or to hydrolysis of the thioester bond. *S*-Citryl-CoA (0.1 μmol) was substituted for 4-malyl-CoA in the normal cleavage assay system. No increase in E_{315} (the $\lambda_{\max}$ of oxaloacetate phenylhydrazine) was recorded even with the addition of up to 20 μg of purified enzyme. Hence the enzyme was not able to cleave citryl-CoA. To test for citryl-CoA hydrolysis, 20 μg of purified enzyme was incubated in the following reaction mixture, containing, in 1 ml, potassium phosphate buffer, pH 7.4 (100 μmol), MgCl₂ (10 μmol), 5,5'-dithiobis-(2-nitrobenzoate) (0.1 μmol), and citryl-CoA (0.1 μmol). No increase in E_{412} was recorded, showing the absence of deacylase activity towards citryl-CoA.

Replacement of 4-malyl-CoA by either equimolar (DL)-3-hydroxybutyryl-CoA or 3-hydroxy-3-methylglutaryl-CoA in the cleavage assay mixture produced no reaction in terms of production of acetyl-CoA and acetaldehyde or acetoacetate respectively.

The specificity of malyl-CoA lyase towards glyoxylate and acetyl-CoA was tested by modifying the mixture described in the Materials and Methods section for discontinuous assay of malyl-CoA synthesis. In the first case glyoxylate was replaced by equimolar quantities of glycollate, glycolaldehyde or pyruvate and the acetyl-CoA concentration was monitored. In the second case acetyl-CoA was replaced by equimolar quantities of propionyl-CoA, *n*-butyryl-CoA, succinyl-CoA or oxalyl-CoA and the glyoxylate concentration was monitored. Glyoxylate

Table 2. Metal ion requirement

Reaction mixtures contained in a final volume of 1.0 ml: *N*-glycylglycine buffer, pH 7.5 (100 μ mol), 4-malyl-CoA (0.2 μ mol), phenylhydrazine-HCl (5 μ mol) and EDTA (0.2 μ mol). The reaction was initiated by the addition of metal ion (10 μ mol) and the E_{324} recorded at 30°C with a reference mixture lacking metal ion. The enzyme preparation contained 0.5 mg of protein/ml.

Metal ion	Rate (μ mol/min per ml of enzyme)	Rate relative to Mg^{2+} (%)
Mg^{2+}	12.9	—
Co^{2+}	12.9	100
Mn^{2+}	11.4	88
Zn^{2+}	5.3	41
Ni^{2+}	1.2	9
Ca^{2+}	0	0

disappeared in the presence of propionyl-CoA at one-tenth of the rate observed in the presence of acetyl-CoA; the product of this slow reaction was not determined. In no other case could any appreciable reaction be detected.

Metal ion requirement

Malyl-CoA lyase was found to have an absolute requirement for a bivalent metal ion. The relative effectiveness of various ions is shown in Table 2.

Inhibition

(2*R*)-4-Malyl-CoA caused inhibition of (2*S*)-4-malyl-CoA cleavage. At equimolar concentrations (0.2 mM each) of the two isomers 28% inhibition was observed, and doubling the concentration of the (2*R*) form to 0.4 mM increased the inhibition to 45%. Activity was also dependent on the nature of the buffer system used; Tris buffers caused 45–50% inhibition at 0.1 M relative to equimolar phosphate at the same pH, whereas activities were 10% higher in 0.1 M-*N*-glycylglycine than in 0.1 M-phosphate. Malyl-CoA lyase was not inhibited by the thiol-group binding reagents *N*-ethylmaleimide, iodoacetate or *p*-chloromercuribenzoate at 1 mM concentrations.

pH optimum

The activity of malyl-CoA lyase was investigated as a function of pH over the range pH 5.5–10.0. Enzyme assays were performed in the direction of 4-malyl-CoA cleavage discontinuously owing to the pH-dependence of the coupled glyoxylate-phenylhydrazine reaction. Phosphate buffer was used

Fig. 2. pH-dependence of malyl-CoA lyase

Activities were measured in 0.1 M-potassium phosphate (Δ) and 0.1 M-*N*-glycylglycine ($\bullet$) buffers. Reaction mixtures contained in 2.0 ml: buffer (100 μ mol), $MgCl_2$ (20 μ mol) and 4-malyl-CoA (0.1 μ mol). The reaction was initiated by the addition of enzyme preparation (1.0 μ g of protein). Samples (250 μ l) were withdrawn at 30 s time-intervals and the reaction was stopped with $HClO_4$. After neutralization, glyoxylate was measured as described in the Materials and Methods section.

between pH 5.0 and 7.8, and *N*-glycylglycine between 7.0 and 10.0. The results are shown in Fig. 2. The enzyme has a broad optimum between pH 7.2 and 8.2 with maximum activity being recorded at pH 7.8.

Determination of the equilibrium constant

Reaction mixtures (3.0 ml) were prepared containing 4-malyl-CoA, acetyl-CoA and glyoxylate at various concentrations in the range 0–0.5 mM, potassium phosphate buffer, pH 7.4 (300 μ mol), $MgCl_2$ (30 μ mol) and enzyme (20 μ g of protein). Samples were withdrawn after 1–1½ h incubation at 30°C, and the concentrations of the three substrates determined after treatment with $HClO_4$ as described above. It had been confirmed in pilot experiments that equilibrium was attained within this time. The results obtained are listed in Table 3.

Determination of Michaelis constants

Michaelis constants were determined from double-reciprocal plots of initial velocity versus substrate concentration (Lineweaver & Burk, 1934) (Table 4).

Table 3. *Determination of the equilibrium constant for the malyl-CoA lyase reaction*

The experimental procedure is described in the text. Malyl-CoA and acetyl-CoA were determined in the same sample, by using citrate synthase. Control mixtures lacking enzyme showed no measurable change in concentrations under the same conditions. K is defined as $\frac{[\text{acetyl-CoA}][\text{glyoxylate}]}{[4\text{-malyl-CoA}]}$.

Expt. no.	Initial substrate concn. (mM)			Final substrate concn. (mM)			K (M)
	Malyl-CoA	Acetyl-CoA	Glyoxylate	Malyl-CoA	Acetyl-CoA	Glyoxylate	
1	0.108	0	0.118	0.042	0.074	0.223	3.9×10^{-4}
2	0	0.525	0.470	0.170	0.328	0.289	5.6×10^{-4}
3	0	0.451	0.445	0.155	0.285	0.289	5.3×10^{-4}
4	0.277	0	0	0.083	0.196	0.187	4.7×10^{-4}
5	0.135	0	0.225	0.051	0.079	0.302	4.7×10^{-4}
6	0.270	0.515	0	0.154	0.620	0.111	4.5×10^{-4}
						Mean	4.7×10^{-4}

Table 4. *Michaelis constants of malyl-CoA lyase*

The concentrations of additional reactants are shown in parentheses. The Michaelis constant for Mg^{2+} was determined in the direction of 4-malyl-CoA cleavage. All the reactions were carried out in 0.1M-potassium phosphate buffer (pH 7.4) at 30°C.

Substrates	K_m (M)
(2S)-4-Malyl-CoA (10mM- Mg^{2+})	6.6×10^{-5}
Acetyl-CoA (10mM-glyoxylate; 10mM- Mg^{2+})	1.5×10^{-5}
Glyoxylate (0.4mM-acetyl-CoA; 10mM- Mg^{2+})	1.7×10^{-3}
Mg^{2+} (0.2mM-4-malyl-CoA)	1.2×10^{-3}

Discussion

An ATP- and CoA-dependent cleavage of malate into acetyl-CoA and glyoxylate has been observed in extracts of *Rhodospseudomonas spheroides* (Tuboi & Kikuchi, 1962, 1963), *Escherichia coli*, *Rhodospirillum rubrum* and rabbit liver (Stern, 1963), *Pseudomonas* MA and *Hyphomicrobium* WC (Hersh & Bellion, 1972; Bellion & Hersh, 1972), *Pseudomonas* MS (Salem *et al.*, 1973), *Hyphomicrobium* X (Harder *et al.*, 1973) and *Bacterium* 5H2 (Cox & Zatman, 1973). There is evidence that in *R. spheroides* (Mue *et al.*, 1964; Tuboi & Kikuchi, 1965) the ATP- and CoA-dependent malate lyase activity is due to the combined action of two separate enzymes: a malate thiokinase and malyl-CoA lyase reaction (1). A similar situation probably also exists in *Pseudomonas* MS and *Hyphomicrobium* X (Salem *et al.*, 1973). Definitive evidence for the presence of these two enzymes in *Pseudomonas* MA has been provided by Hersh (1973), who has separated and purified each from the methylamine-grown organism. *Pseudomonas* AM1 and the methane-utilizing bacteria *Pseudomonas methanica*, *Methylococcus capsulatus* and *Methylosinus trichosporium* differ from the above

organisms in possessing malyl-CoA lyase but not an ATP- and CoA-dependent malate thiokinase (Salem *et al.*, 1973). In the present work, malyl-CoA lyase from methanol-grown *Pseudomonas* AM1 has been purified essentially to homogeneity, as judged by ultracentrifugal and electrophoretic analysis. It is a relatively stable enzyme with a molecular weight of 190000.

As far as has been tested, the enzyme is specific for cleavage of (2S)-4-malyl-CoA or condensation of glyoxylate with acetyl-CoA or, to a lesser extent, propionyl-CoA. Citrate synthase is known to cleave malyl-CoA to acetyl-CoA and glyoxylate (Eggerer *et al.*, 1964); however, the absence of any citrate synthase activity in the lyase from *Pseudomonas* AM1 eliminates the possibility that the latter could in fact be a citrate synthase. Similarly, the lack of malate synthase activity in the lyase from *Pseudomonas* AM1 eliminates the possibility that the latter could be a malate synthase, which, unlike other malate synthases (Dixon *et al.*, 1960; Dixon & Kornberg, 1962; Eggerer *et al.*, 1964), is able to cleave malyl-CoA. Activity of malyl-CoA lyase was completely dependent on the presence of a bivalent metal ion, Mg^{2+} or Co^{2+} giving the highest activity.

The equilibrium constant for reaction (1) was determined to be 4.7×10^{-4} M at 30°C; this corresponds to a value for ΔG° of +19.2 kJ (+4.6 kcal). A value of +14.7 kJ (+3.5 kcal) may be calculated for this reaction by assuming the values of ΔG_f° for glyoxylate, acetate and malate, as given by Burton (1961), and assuming that $\Delta G'$ for the hydrolysis of malyl-CoA:

is -37.2 kJ (-8.9 kcal) at pH 7.5, as is the case for hydrolysis of acetyl-CoA (Burton, 1961). The equilibrium position of reaction (1) is such that at millimolar concentrations of substrates substantial reaction in either direction is feasible, although,

physiologically, the reaction is thought to proceed in the direction of cleavage, as in reaction (1). However, it should be noted that an organism possessing malyl-CoA lyase and a malyl-CoA deacylase or transferase could then perform the same overall reaction as that catalysed in one step by malate synthase.

The malyl-CoA lyases from *Pseudomonas* AM1 and *Pseudomonas* MA (Hersh, 1973) are the first enzymes that have been demonstrated to catalyse a readily reversible reaction of the type:

In the direction left to right, similar types of reactions are catalysed by citramalyl-CoA lyase (Wang *et al.*, 1961; Lardy, 1969) and 3-hydroxy-3-methylglutaryl-CoA lyase (EC 4.1.3.4) (Stegink & Coon, 1968). In the first case no evidence could be found for reversibility and in the second case the reaction with the enzyme from bovine liver is stated to be essentially irreversible. In comparing these enzymes with malyl-CoA lyase it may be recalled that the latter enzyme is dependent on bivalent metal ions for activity and is unaffected by thiol-binding reagents. The activity of citramalyl-CoA lyase is apparently independent of the presence of metal ions, whereas 3-hydroxy-3-methylglutaryl-CoA lyase is dependent on bivalent metal ions and is sensitive to thiol-binding reagents. A further analogous reaction to that catalysed by malyl-CoA lyase is the cleavage of citryl-CoA to acetyl-CoA and oxaloacetate catalysed by citrate synthase (Eggerer & Remberger, 1963a) and ATP citrate lyase (EC 4.1.3.8) (Eggerer & Remberger, 1963b), although, of course, this reaction is only a partial reaction with respect to the overall physiological reactions catalysed by the enzymes.

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