Fat Metabolism in Higher Plants. XVII. Metabolism of Malonic Acid and Its α-Substituted Derivatives in Plants [1, 2]

M. D. Hatch [3] & P. K. Stumpf

Department of Biochemistry & Biophysics, University of California, Davis

It is now established that malonic acid is an important metabolite of plants and other tissues. Malonyl-CoA[4] is an intermediate in the synthesis of fatty acids by preparations from plants (1) and many other tissues (17). It has been known for some time that certain aromatic compounds of plants and moulds are synthesized by a more or less direct condensation of acetate units (14). Recent studies with molds indicate that malonyl-CoA is an intermediate in this process, acting in much the same way as it does in fatty acid synthesis to introduce acetate units into aromatic compounds (3, 11), and related cyclic compounds (3). This is presumptive evidence that the same process is involved in the synthesis of plant aromatic compounds not derived from shikimic acid. Free malonic acid accumulates in many plants (2), while plant tissues and extracts convert labelled malonic acid to CO_2 and Krebs cycle acids (4, 15, 21). There is evidence that the dissimilation of malonate involves its conversion to the CoA derivative, followed by decarboxylation to acetyl-CoA and CO_2 (4, 21). Decarboxylases of this type have been isolated from bacteria (8, 20) and from animal tissues (13).

The carboxylation of acetyl-CoA to yield malonyl-CoA apparently is involved in the synthesis of fatty acids from C^{14}-acetate by avocado preparations (1). A similar enzyme has been implicated in fatty acid synthesis by mammalian enzyme systems. However, the only mammalian acyl-CoA carboxylases which have been described in detail (10, 18) utilize acetyl-CoA at only 1/100 the rate of propionyl-CoA. Recently, we described an acyl-CoA carboxylase from wheat germ which was most active with acetyl-CoA (6, 7). Other acyl-CoA compounds were also carboxylated, the overall reaction being described in equation I.

$$R\text{-}CH_2\text{-}COSCoA + CO_2 \underset{\text{Biotin}}{\overset{\text{ATP, } Mg^{++}}{\rightleftharpoons}} \underset{|}{\overset{COOH}{}}R\text{-}CH\text{-}COSCoA \qquad \text{I}$$

During these studies it was found that the same enzyme catalyzed a separate but related reaction. This reaction, which required no added cofactors, involved a reversible transcarboxylation from malonyl-CoA or its α-substituted derivatives to an acyl-CoA acceptor according to equation II.

$$R\text{-}CH_2\text{-}COSCoA + \overset{COOH}{\underset{|}{CH_2}}\text{-}COSCoA \underset{\text{Biotin}}{\rightleftharpoons} R\text{-}\overset{COOH}{\underset{|}{CH}}\text{-}COSCoA + CH_3\text{-}COSCoA \qquad \text{II}$$

In this paper are described further studies of the metabolism of malonic acid and its α-substituted derivatives by wheat germ enzymes. A survey of the activity of acetyl-CoA carboxylase, transcarboxylase, malonic thiokinase, malonyl-CoA thioesterase, and malonyl-CoA decarboxylase in a variety of plant tissue extracts was conducted.

Materials & Methods

Wheat germ was obtained from General Mills, San Francisco. Rice germ was a gift from Dr. S. Grisola of the University of Kansas. The seed variety of these tissues was not known. The following plants were used as a source of tissues for the survey of enzyme activities: rice (*Oryza sativa* L.); peanut (*Arachis hypogea* L.); soy bean (*Glycine maxima* L.); bean (*Phaseolus vulgaris* L.); wheat (*Triticum vulgare* Vill.); safflower (*Carthamus tinctorius* L.); barley (*Hordeum vulgare* L.); spinach (unknown variety); sweet clover (*Melilotus alba* Desr.); alfalfa (*Medicago sativa* L.) lupin (*Lupinus albus* L.); avocado (*Persea americana* L.). Sweet clover, alfalfa, spinach, and bean leaves were from mature plants. All the remaining tissues were obtained from plants germinated for 5 to 7 days in damp vermiculite. Seeds were soaked for 10 hours in distilled water prior to the preparation of acetone powders.

▶ Chemicals & Preparation of Substrates: The commercial source of most of the biochemicals and labelled compounds used have been described (7). Propionate-1-C^{14} (Research Specialties Co., Rich-

---

[1] Received July 20, 1961.

[2] Supported in part by a grant from the National Science Foundation (G-14823).

[3] Fulbright Travel Fellow 1959-61. Present address: David North Plant Research Centre, C.S.R. Co. Ltd., 52 Meiers Road, Indooroopilly, Queensland, Australia.

[4] The following abbreviations are used: CoA, coenzyme A; ATP, adenosinetriphosphate; EDTA, ethylenediamine tetra-acetic acid; ADP, adenosinediphosphate; TPNH, reduced triphosphopyridine nucleotide; tris, tris (hydroxmethyl) aminomethane.

mond, Cal.), butyrate-1-C^{14} (Volk Radiochemical Co.) and methylmalonate-1-C^{14} (a gift from Dr. M. Flavin) were also used. The methods employed to prepare, purify, and estimate acyl-CoA substrates have been described (7). Methylmalonyl-1-C^{14}-CoA (labelled specifically in the ester carboxyl group) was prepared enzymically from propionate-1-C^{14} using wheat germ thiokinase and carboxylase preparations. The product was purified by paper chromatography (16), then eluted from the developed chromatograms.

► Preparation of Enzymes: The preparation of wheat germ acetone powder, and subsequent extraction, centrifugation, and protamine sulfate treatment, were as previously described (7). The enzyme fraction used for preliminary studies was prepared by treating the protamine sulfate supernatant with saturated $(NH_4)_2SO_4$ (pH 7.0, 0° C) to 80 % saturation with respect to $(NH_4)_2SO_4$. The precipitated protein was centrifuged, dissolved in 0.01 M K_2HPO_4, then dialysed with stirring against 4 liters of the same buffer for 8 hours at 4 C. A similar procedure was used to prepare $(NH_4)_2SO_4$ subfractions which precipitated between 0 % to 28 %, 28 % to 36 %, 36 % to 45 %, 45 % to 54 %, and 54 % to 65 % saturation.

For the survey of enzyme activities, tissue extracts were prepared in the following manner: tissues were washed thoroughly with distilled water then homogenized with 20 volumes of acetone (−15 C). The residual material was filtered, washed in cold acetone, then dried under vacuum. Two grams of each acetone powder were extracted for 30 minutes with 25 ml of 0.1 M K_2HPO_4 (pH 8.3) containing 3×10^{-4} M EDTA, then centrifuged at 35,000 × *g* for 30 minutes. The precipitated material was re-extracted with another 15 ml of the same buffer and centrifuged. The centrifugation and subsequent steps were carried out at 0° C. Saturated $(NH_4)_2SO_4$ was added to the combined soluble extracts to obtain the protein which precipitated between 0 % and 75 % saturation. The precipitated protein was dissolved in 0.01 M K_2HPO_4 buffer, pH 8.3, and dialysed as previously described. The protein content of the various enzyme extracts was determined spectrophotometrically (19).

► Enzymatic assays: Except where otherwise indicated reaction mixtures for the various enzyme assays were, A: Carboxylase and transcarboxylase: reaction mixture components, methods of assay, and identification of reaction products were as previously described (7). B: Thiokinase: enzyme [45 %–54 % $(NH_4)_2SO_4$ fraction], 2 mg; malonate-1,3-C^{14}, 0.05 μmoles (210,000 dpm); ATP, 4 μmoles; $MgCl_2$, 8 μmoles; CoA, 0.2 μmoles; tris-HCl buffer, 50 μmoles (pH 8.0). Total volume was 1.0 ml. C: Thioesterase: enzyme (45 %–54 % $(NH_4)_2SO_4$ fraction), 2 mg; malonyl-1,3-C^{14}-CoA, 0.1 μmole (220,000 dpm); tris-HCl buffer, 50 μmoles (pH 8.0). Total volume was 1.0 ml. D: Decarboxylase: enzyme (28 %–36 % $(NH_4)_2SO_4$ fraction), 3 mg; malonyl-1,3-C^{14}-CoA, 0.1 μmole (220,000 dpm); potassium phosphate buffer, 50 μmoles (pH 7.5). Total volume was 1.0 ml. All assays were conducted at 30 C.

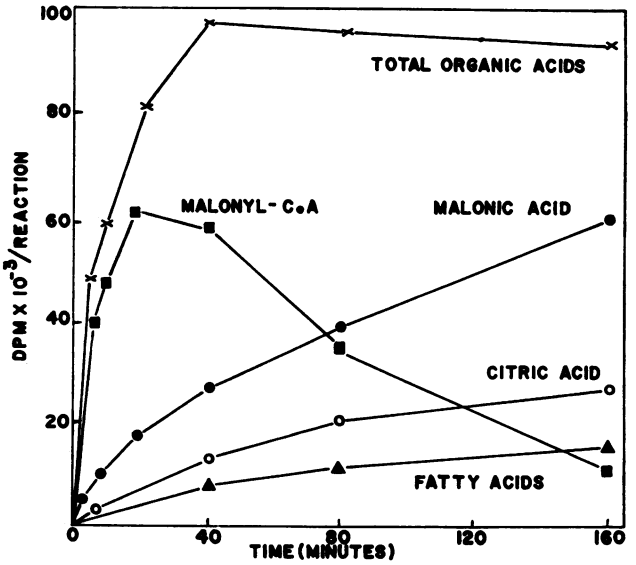
After the specified incubation periods reaction mixtures were treated with either 0.3 ml of concentrated NH_4OH or 0.2 M hydroxylamine (pH 8.0). These reagents formed, respectively, the monoamides or the monohydroxamates of the CoA derivatives present in reaction mixtures. Organic acids and the derivatives of the CoA compounds were extracted from reaction mixtures with acetone as previously described (7). Total non-volatile radioactivity was determined by plating aliquots of the acetone extract and counting with a Nuclear-Chicago gas flow counter at infinite thinness. Reaction products were identified, as previously described (7), by co-chromatography on paper with authentic compounds using various eluting solvents. The contribution of individual reaction components to the total radioactivity was determined by strip-counting of developed chromatograms, followed by measurement of peak areas with a planimeter.

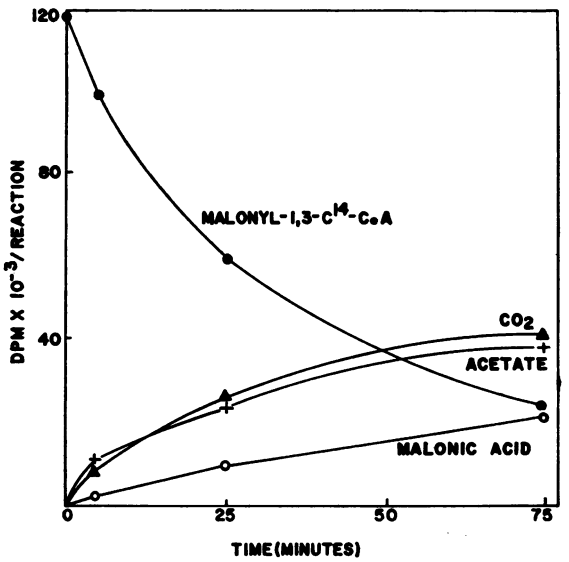
$C^{14}O_2$ was trapped from reaction mixtures in 20 % KOH, transferred to the hydroxide of hyamine-10X, then aliquots counted in a Tricarb liquid scintillation counter. The phosphor solution consisted of 0.6 % 2,5-diphenyloxazole and 0.05 % 1,4-di[2-(5-phenyloxazolyl)] benzene in toluene. Labeled fatty acids were identified by paper chromatography (12) and by gas chromatography of their methyl esters with authentic marker compounds.

Results

► Malonic Acid Metabolism in a Soluble Wheat Germ Extract: For these preliminary studies the 0 % to 80 % $(NH_4)_2SO_4$ fraction was used. When supplemented with ATP, $MgCl_2$, CoA, and TPNH, this enzyme preparation catalyzed the incorporation of acetate-1-C^{14} into malonyl-CoA, free malonic acid and fatty acids (fig 1). The results were consistent with malonyl-CoA being a precusor of malonic acid and fatty acids. The fatty acids, predominantly a mixture of palmitate and stearate, were found in the phospholipid fractions. In this system some label appeared in citric acid. Similar results were obtained when $C^{14}O_2$ and unlabeled acetate were added, except that fatty acids were not labeled. These results are consistent with the operation of an acetic thiokinase, an acetyl-CoA carboxylase and a malonyl-CoA thioesterase.

When malonic acid-1,3-C^{14} was supplied with CoA, ATP, and $MgCl_2$, labeled malonyl-CoA and CO_2 were formed. Malonic thiokinase and probably malonyl-CoA decarboxylase were responsible for these conversions. The operation of malonyl-CoA decarboxylase was confirmed by adding malonyl-1,3-C^{14}-CoA (fig 2). The disappearance of malonyl-CoA, other than that converted to free malonic acid, was accompanied by the quantitative formation of $C^{14}O_2$. Probably due to acetyl-CoA thioesterase activity, acetyl-CoA was not detected but labeled acetate





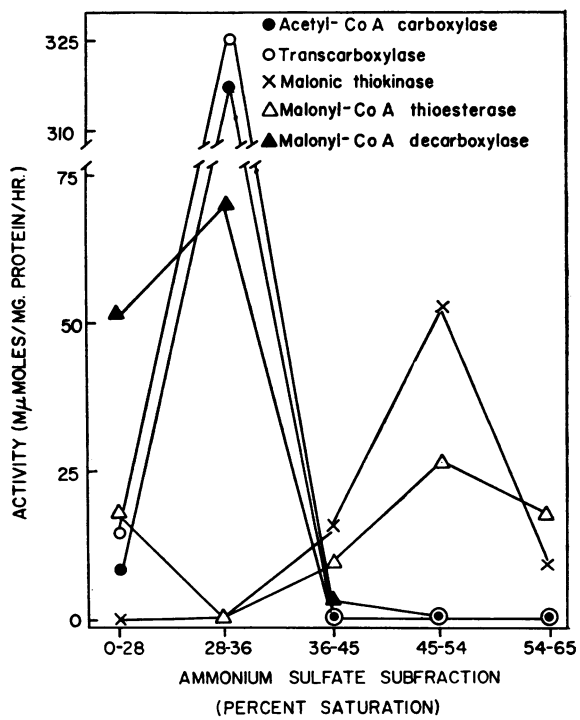


Fig. 1. Incorporation of acetate-1-C^{14} into malonyl-CoA, malonic acid, and fatty acids. Reaction mixtures contained wheat germ 0 % to 80 % $(NH_4)_2SO_4$ fraction (14 mg of protein); acetate-1-C^{14}, 0.3 μmoles (500,000 dpm); CoA, 0.2 μmoles; ATP, 10 μmoles; $MgCl_2$, 2 μmoles; TPN, 0.25 μmoles; glucose-6-phosphate, 1.5 μmoles in 0.05 M potassium phosphate buffer, pH 8.0. The total volume was 1.0 ml.

Fig. 2. Breakdown of malonyl-1,3-C^{14}-CoA to CO_2, acetate and free malonic acid. Reaction mixtures contained wheat germ 0 % to 80 % $(NH_4)_2SO_4$ fraction (8 mg of protein), and malonyl-1,3-C^{14}-CoA, 0.16 μmoles, (120,000 dpm) in 0.05 M potassium phosphate buffer, pH 7.5. The total volume was 1.0 ml. The values for acetate were determined by difference since it could not be quantitatively recovered.

Fig. 3. Partial fractionation of enzymes involved in malonic acid metabolism with $(NH_4)_2SO_4$. Assay procedures for the various enzymes were as described in the Methods section.

was identified by chromatography. The curve for acetate was calculated by difference.

► Fractionation of Wheat Germ Extract With $(NH_4)_2SO_4$: Fractionation of crude extracts with $(NH_4)_2SO_4$ resulted in partial or complete separation of the activities observed in the 0 % to 80 % $(NH_4)_2SO_4$ fraction (fig 3). The 45 % to 54 % $(NH_4)_2SO_4$ fraction was used for further studies of thiokinase and thioesterase activities and the 28 % to 36 % $(NH_4)_2SO_4$ fraction for decarboxylase.

► Carboxylase and Transcarboxylase: Carboxylase and transcarboxylase activities were localized almost entirely in the 28 % to 36 % $(NH_4)_2SO_4$ fraction which was contaminated only by malonyl-CoA decarboxylase (fig 3). As already described (7), these activities were purified further to remove decarboxylase. Malonyl-CoA, methylmalonyl-CoA and ethylmalonyl-CoA were formed from acetyl-CoA, propionyl-CoA, and butyryl-CoA, respectively, by carboxylase or transcarboxylase.

Table I

Thiokinase Activity of Wheat Germ 45 % to 54 % $(NH_4)_2SO_4$ Fraction With Different Substrates

Substrate*	CoA Derivative formed mμmoles/mg protein/hour
Acetate-1-C^{14}	27
Propionate-1-C^{14}	8.3
Butyrate-1-C^{14}	5.1
Malonate-1-C^{14}	38
α-Methylmalonate-1-C^{14}	8.4

* The amount of substrates added was acetate-1-C^{14}, 0.1 μmoles (750,000 dpm); propionate-1-C^{14}, 0.1 μmole (1,100,000 dpm); butyrate-1-C^{14}, 0.1 μmole (220,000 dpm); malonate-1-C^{14}, 0.05 μmole (210,000 dpm) and α-methylmalonate-1-C^{14}, 0.6 μmole (25,200 dpm) in reaction mixtures as described in the Methods section.

► Thiokinase: ATP and Mg^{++} were essential for thiokinase activity and the pH optimum with malonate as substrate was approximately 8.0. Because of the importance of acetyl-, propionyl-, and butyryl-CoA as substrates for carboxylase and transcarboxylase, the formation of these compounds by wheat germ thiokinase was examined (table I). In this table the activity of the 45 % to 54 % $(NH_4)_2SO_4$ fraction toward monocarboxylic acids was compared with that for malonic and methylmalonic acids. It should be pointed out that these would be minimal values since the enzyme fraction also contained thioesterase activity.

► Thioesterase: Wheat germ contained two malonyl-CoA thioesterases (fig 3). The malonyl-CoA thioesterase activity of the 0 % to 28 % $(NH_4)_2SO_4$ fraction was accompanied by a much higher acetyl-CoA thioesterase activity. The 45 % to 54 % $(NH_4)_2SO_4$ fraction, which contained the major proportion of malonyl-CoA thioesterase activity, was relatively inactive toward acetyl-CoA. The thioesterase activity of this fraction apparently had a higher specificity for malonyl-CoA. This enzyme had a pH optimum between 8.0 and 8.5.

► Decarboxylase: There was conclusive evidence that the decarboxylase activity of wheat germ extracts was not due to reversal of the carboxylase reaction. The two activities were partially separated by $(NH_4)_2SO_4$ fractionation (fig 3). In contrast to the carboxylase reaction, the decarboxylase did not require ADP and Mg^{++}, was not inhibited by thiol reagents or avidin, and was irreversible. The overall stoichiometry of the reaction is shown in table II. Since the 28 % to 36 % $(NH_4)_2SO_4$ fraction contained some acetyl-CoA thioesterase activity, it was not possible to recover acetyl-CoA quantitatively as such. However, when reaction mixtures were supplemented with oxalacetate, acetyl-CoA was trapped quantitatively as citric acid.

► Further Studies of Metabolism of α-Substituted Malonic Acid Derivatives: Since α-substituted malonic acid derivatives were formed by the action of wheat germ carboxylase or transcarboxylase, it was of interest to examine other metabolic activities of these compounds or their CoA derivatives. The activity of wheat germ thiokinase with methylmalonate, and of methylmalonyl-CoA with thioesterase and decarboxylase, was examined (table III). Because of the difficulty in obtaining quantities of methylmalonyl-CoA by enzymic synthesis, only small amounts (8×10^{-6} M, final concentration) were added to reaction mixtures. It is probable that the rate of thioesterase and decarboxylase action with methylmalonyl-CoA would be greater at higher substrate concentrations.

► Survey of Enzymes Involved in Malonic Acid Metabolism: To assess the general importance of the various enzymes described, their activity in extracts of a variety of plant tissues was determined (table IV). Except where otherwise indicated, thioesterase and decarboxylase activities were not affected by interfering enzymes. However, the values for carboxylase, transcarboxylase, and thiokinase activities should be regarded as minimal, since they would be reduced by thioesterase and decarboxylase activity. The ratio of carboxylase to transcarboxylase activity varied, presumably due to different degrees of interference by thioesterase and decarboxylase. Although carboxylase and transcarboxylase had a relatively wide distribution some significant trends were observed. The results suggested that these activities may be concentrated in seed embryos since wheat and

Table II

Stoichiometry of Malonyl-CoA Decarboxylase Reaction

Zero time*		40 Min		
Substrate	Amount mμmoles	Malonyl-CoA decomposed mμmoles	CO_2 formed mμmoles	Acetyl-CoA formed mμmoles
Malonyl-1,3-C^{14}-CoA	98	74	68	23**
Malonyl-1,3-C^{14}-CoA + oxalacetate	98 2,000	70	66	72***

* Reaction mixtures and assay procedures were as described in the Methods section.

** Measured chromatographically as acetyl-hydroxamate formed by adding hydroxylamine at the end of the reaction.

*** Calculated from the amount of labeled citric acid formed [the condensing enzyme was present in the 28 % to 36 % $(NH_4)_2SO_4$ fraction].

Table III

Activity of Wheat Germ Thiokinase, Thioesterase, & Decarboxylase With α-Methylmalonate or Its CoA Derivative

Enzyme assayed	Substrate added	Product formed mμmoles/reaction
Thiokinase	Methylmalonate-1-C^{14}, 500 mμmoles (21,000 dpm)	69*
Thioesterase	Methylmalonyl-1-C^{14}-CoA, 8 mμmoles (74,000 dpm)	3.2**
Decarboxylase	Methylmalonyl-1-C^{14}-CoA, 8 mμmoles (74,000 dpm)	6.8***

Reaction mixtures and assay methods are described in the Methods section. The $(NH_4)_2SO_4$ sub-fractions used were 43 % to 54 % for thiokinase (8 mg protein), 43 % to 54 % for thioesterase (4 mg protein) and 28 % to 36 % for decarboxylase (3 mg protein). Reaction times were 60 minutes for thiokinase and 20 minutes for thioesterase and decarboxylase.

* Methylmalonyl-CoA
** Methylmalonate
*** CO_2 and propionyl-CoA

Table IV

Survey of Enzymes Involved in Malonic Acid Metabolism

Tissue*	Enzyme activity** (mμmoles/g acetone powder/min)				
	Carboxylase	Transcarboxylase	Thiokinase	Thioesterase	Decarboxylase
Wheat germ	45	56	14	12	19
Rice germ	27	34	19	11	14
Peanut seed	2.8	2.1	5.8	19	19
Soy bean seed	7.5	16	0	41	49
Bean seed	4.3	6.5	16	4.2	11.5
Wheat cotyledon	0.9	2.0	3.6	2.9	2.1
Safflower "	0	4.3	29	>140	0 ?
Peanut "	0	0	7.2	3.1	4.1
Soy bean "	4.8	4.8	2.0	24	35
Bean "	2.5	5.6	1.0	6.1	0.4
Barley epicotyl	8.8	17	37	86	63
Spinach leaf	8.1	7.5	13	17	10
Clover "	12	16	7.2	3.4	13
Alfalfa "	7.3	24	14	>78	14
Bean "	6.5	12	6.3	3.1	19
Rice roots	0.6	1.6	2.1	6.1	0.4
Barley "	0	0	2.8	3.9	0.3
Lupin "	0	0	0	4.4	1.8
Pea seedling	0.4	2.5	4.2	7.0	3.8
Avocado mitochondria	2.1	1.2	5.8	12	4.6

* The extraction of tissue acetone powders, the reaction mixtures, and the assay methods are described in the Methods section.
** Each assay system contained 5 mg of protein.

rice germ were most active, while extracts from whole seeds showed only low activity. Both leguminous and non-leguminous leaves were an active source of these enzymes, but negligible activity was found in root tissues. While no significant trends were observed with thiokinase, thioesterase, and decarboxylase, all were widely distributed.

Discussion

The results presented indicate that enzymes necessary for the control of malonic acid metabolism occur in plants. Since intercellular localization of these enzymes was not examined, the extent to which this factor might influence their interaction is not known. The balance of such interaction, to whatever degree it occurs, would be of importance in determining, A: the rate of synthetic processes which require malonyl-CoA, such as the synthesis of fatty acids and aromatic compounds, B: the extent to which free malonic acid accumulates in plants or is decarboxylated to acetate and oxidized via the Krebs cycle, C: the rate at which propionate is catabolized by the β-oxidation pathway described by Giovanelli and Stumpf (4). The interaction of these enzymes, to control the metabolism of malonic acid and α-substituted malonic acid derivatives, is shown schematically in figure 4. The α-substituted malonic acid derivatives have not previously been implicated in plant metabolism and to our

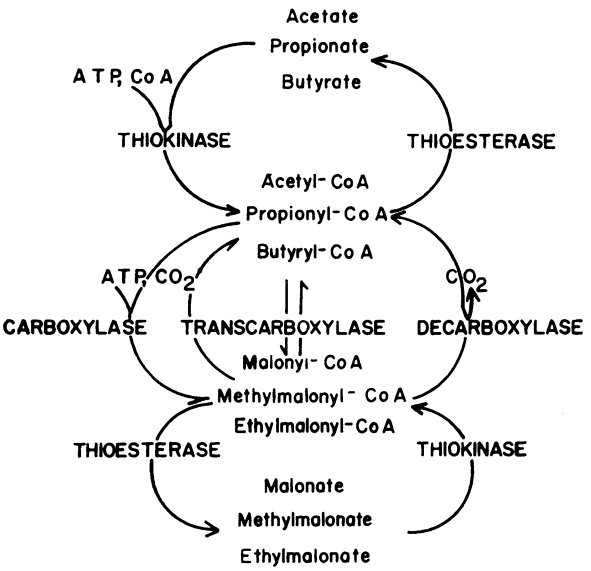


Fig. 4. Synthesis and breakdown of malonic acid and its α-substituted derivatives in plants.

knowledge have not been reported to occur in plant tissues. Possible functions for these compounds in the synthesis of branched chain fatty acids and substituted aromatic compounds have been discussed previously (7).

Although acetyl-CoA carboxylase is widely distributed in plants, there is evidence that malonic acid might be formed by other pathways in some tissues. Mitochondria from certain plant tissues oxidize propionate by a pathway which involves malonyl-CoA as an intermediate (4). Recently, Huffaker and Wallace (9) briefly described a bean root system which apparently synthesized malonic acid by yet another route. Oxalacetate was implicated as a precursor of malonic acid in this process.

Summary

An acyl-CoA carboxylase from wheat germ, which formed malonyl-CoA and its α-substituted derivatives, was previously described. The same enzyme was shown to catalyze ATP-independent transcarboxylations. The present communication describes the preparation and some of the properties of a malonic thiokinase, a malonyl-CoA thioesterase, and a malonyl-CoA decarboxylase from wheat germ. These enzymes were also active with methylmalonate or its CoA derivative. In view of their importance as carboxylase and transcarboxylase substrates, it was of interest that the CoA derivatives of acetate, propionate, and butyrate were formed by thiokinases present in wheat germ.

The activity of these enzymes, including the carboxylase and transcarboxylase, was examined in a wide variety of plant tissue extracts. This survey indicated that enzymes for the synthesis and breakdown of malonic acid are widely distributed. The possible role of these enzymes in the control of metabolic processes, and of methylmalonate as a metabolite, is discussed.

Literature Cited

1. Barron, E. J., C. Squires, and P. K. Stumpf. 1961. Fat metabolism in higher plants. XV. Enzymic synthesis of fatty acids by an extract of avocado mesocarp. J. Biol. Chem. 236: 2610–2614.
2. Bentley, L. E. 1952. Occurrence of malonic acid in plants. Nature 170: 847–848.
3. Bentley, R., & J. G. Keil. 1961. The role of acetate & malonate in the biosynthesis of penecillic acid. Proc. Chem. Soc. (London). March pp. 111.
4. Giovanelli, J. & P. K. Stumpf. 1957. Oxidation of malonate by peanut mitochondria. Plant Physiol. 32: 498–499.
5. Giovanelli, J. & P. K. Stumpf. 1958. Fat metabolism in higher plants. X. Modified β-oxidation of propionate by peanut mitochondria. J. Biol. Chem. 231: 411–426.
6. Hatch, M. D. & P. K. Stumpf. 1961. Synthesis & breakdown of malonyl-CoA by wheat germ enzymes. Federation Proc. 20: 273.
7. Hatch, M. D. & P. K. Stumpf. 1961. Fat metabolism in higher plants. XVI. Acetyl-CoA carboxylase & acetyl-CoA-malonyl-CoA transcarboxylase from wheat germ. J. Biol. Chem. 236: 2879–2885.
8. Hayaishi, O. 1955. Enzymic decarboxylation of malonic acid. J. Biol. Chem. 215: 125–136.
9. Huffaker, R. C. & A. Wallace. 1961. Malonate synthesis via dark CO_2 fixation in bush-bean roots. Biochem. Biophys. Acta. 46: 403.
10. Lane, M. D., D. R. Halenz, D. P. Kosow, & C. S. Hegre. 1960. Further studies on mitochondrial propionyl carboxylase J. Biol. Chem. 235: 3082–3086.
11. Lynen, F. 1961. Biosynthesis of fatty acids. Federation Proc. 20: paper 2441 (symposium).
12. Mangold, H. K., B. G. Lamp, & H. Schlenk. 1955. Indicators for paper chromatography of lipids. J. Am. Chem. Soc. 77: 6070–6072.
13. Nakada, H. I., B. B. Britton, & J. B. Wolfe. 1957. Degradation of malonic acid by rat tissues. J. Biol. Chem. 226: 145–152.
14. Neish, A. C. 1960. Biosynthetic pathways of aromatic compounds. Ann. Rev. Plant Physiol. 11: 55–80.
15. Shannon, L. M., R. H. Young, & C. Dudley. 1959. Malonate metabolism by plant leaves. Nature. 183: 683.
16. Stadtman, E. R. 1957. Preparation & assay of acetyl-CoA & other thiol esters: Use of hydroxylamine. In: Methods in Enzymology, S. P. Colowick, & N. O. Kaplan, eds. Academic Press Inc., N. Y. Volume 3: 931–941.
17. Stumpf, P. K. 1960. Lipid metabolism. Ann. Rev. Biochem. 29: 261–294.
18. Tietz, A. & S. Ochoa. 1959. Metabolism of propionate in animal tissues. V. Purification & properties of propionyl carboxylase. J. Biol. Chem. 234: 1394–1400.
19. Warburg, O. & W. Christian. 1941. Isolieurung und kristallisation des garungsferments enolase. Biochem. Z. 310: 384–421.
20. Wolfe, J. B. & S. C. Rittenberg. 1954. Malonate decarboxylation by *Pseudomonas fluorescens*. III. The role of acetyl-CoA. J. Biol. Chem. 209: 885–892.
21. Young, R. H. & L. M. Shannon. 1959. Malonate as a participant in the metabolism of bush bean leaves. Plant Physiol. 34: 149–152.