Enzymic Decarboxylation of Oxalate by Extracts of Plant Tissue [1]

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Oxalic acid is widely distributed in plants. Although its concentration is not normally high, certain plants such as Oxalis sp., Begonia sp., rhubarb, and spinach contain large amounts of the acid. Cotyledons of spinach, for example, have been reported to contain up to 26% dry weight of oxalic acid (4).

The only enzyme system previously reported to metabolize oxalate in plants is oxalate oxidase, which catalyzes the reaction:

$$COOH\text{-}COOH + O_2 \rightarrow 2CO_2 + H_2O_2$$

Oxalate oxidase is widely distributed in plants and has been demonstrated to be associated with the plastid fraction in leaves (1, 15) and with a particulate fraction (probably mitochondria) in beet root (11). The physiological significance of this enzyme in plants is unknown.

This report describes the properties of an enzyme system in plant extracts which catalyzes an ATP- and CoA-dependent decarboxylation of oxalate to formate. A preliminary report of this work has appeared (3).

Materials and Methods

The enzyme system was prepared in 2 ways. In early studies the enzyme system was prepared from cotyledons of germinating peas as follows: Peas (*Pisum sativum,* var. Telephone) were germinated in moist peat moss for 5 days. The cotyledons were homogenized with twice their weight of a solution containing 0.45 M sucrose, 0.4 M tris chloride pH 7.2 and 5×10^{-4} M 2,3-dimercapto-l-propanol. The homogenate was squeezed through cheese cloth, the filtrate centrifuged at 1200 × *g* for 7 minutes, and the supernatant solution so obtained centrifuged at 10,000 × *g* for 30 minutes. This crude supernatant fraction contained virtually all the decarboxylase activity of the homogenate. Further centrifuging at 145,000 × *g* for 1 hour showed that the enzyme was not associated with microsomal particles. In some experiments the crude supernatant fraction was adjusted to pH 6.0 with 1 M acetic acid and centrifuged at 10,000 × *g* for 10 minutes. The clear supernatant material was brought to pH 7.2 with 1 M KOH and dialyzed for 3 hours against 0.01 M tris chloride at pH 7.2. The enzyme system so obtained is designated pH 6 supernatant material.

Later studies showed that the enzyme system could be prepared more conveniently from a defatted powder of pea seeds. Pea seeds (*Pisum sativum,* var. Telephone) were powdered in a laboratory seed mill. Pea powder (60 g) was either blended in a Waring Blendor with 1 liter of acetone at −15°, or stirred with 1 liter of ether at room temperature. The defatted powder was separated from the organic solvent by vacuum filtration. Extraction with solvent was repeated 3 times. The resulting powder was dried at room temperature under vacuum. Extracts were prepared by stirring 15 g of defatted powder for 1 hour with 50 ml of 0.001 M glutathione. The slurry was centrifuged at 10,000 × *g* for 10 minutes and the clear supernatant (designated defatted extract) used as a source of enzyme.

Divalent cations were removed from the supernatant fraction with Chelex resin, obtained from Bio-Rad Laboratories, Richmond, California. Chelex (8 g), previously equilibrated with potassium phosphate pH 7.2, was stirred for 30 minutes with 20 ml of the crude supernatant fraction. The slurry was centrifuged, and the supernatant solution (designated Chelex-treated enzyme) used to demonstrate the requirement for divalent cations.

Endogenous anions in the supernatant fraction were removed by passing 50 ml of the supernatant fraction, previously dialyzed for 2 hours against 4 liters of water, through a column (25 mm diameter × 190 mm) of Deacidite FF. Deacidite FF was obtained from The Permutit Company, Sydney, Australia. The resin was used in the fluoride form because of the low affinity of fluoride for anion exchange resins (9). Fluoride at a concentration of 2.5×10^{-2} M had no significant effect on the rate of oxalate decarboxylation.

Preparations catalyzing the activation of oxalate were obtained from defatted extracts of pea seeds by collecting the protein precipitating between zero and 50% saturation with solid ammonium sulfate. This fraction was dissolved in a minimal amount of 0.01 M potassium phosphate buffer pH 7.5 and dialyzed for 3 hours against the same buffer. The dialyzed preparation was clarified by centrifuging at 10,000 × *g* for 10 minutes.

With the exception of a defatted pea powder prepared with ether, enzyme systems were maintained between 0° and 5° during their preparation. All enzyme reaction mixtures were incubated at 30°. Protein in preparations obtained from cotyledons of germinating peas was determined by the biuret method (10). Protein in preparations obtained from defatted seed powders was determined from its absorption at 280 mμ and 260 mμ (10).

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The rate of decarboxylation of oxalate was determined by measuring the rate of release of $C^{14}O_2$ from oxalate-1,2-C^{14}. $C^{14}O_2$ was absorbed by 0.2 ml of 20% KOH in the center well of a conventional Warburg vessel. At the end of the incubation period 0.5 ml of saturated potassium bisulfate was tipped from the side arm. This procedure stops the reaction and liberates $C^{14}O_2$ retained in solution as the bicarbonate ion. Incubation was continued for an additional 10 minutes to allow complete diffusion of $C^{14}O_2$ into the KOH in the center well. $C^{14}O_2$ was precipitated as barium carbonate and counted as an infinitely thin film.

The products of the reaction were determined as follows. Reaction mixtures were extracted continuously with ether for 20 hours, and the ether extract evaporated to a small volume under a flow of ammonia gas. The concentrated extract was made up to a known volume and aliquots assayed for formate-C^{14} and oxalate-C^{14} as follows. To the aliquot was added 5 μmoles of carrier formate and 2.5 μmoles of carrier oxalate. Formate-C^{14} was first estimated by degradation to $C^{14}O_2$ by mercuric acetate (12). Separate experiments demonstrated that this procedure permitted quantitative recoveries of formate-C^{14} with only slight (up to 2%) degradation of oxalate-C^{14}. Oxalate-C^{14} was then estimated by degradation to $C^{14}O_2$ by ceric sulfate (17). $C^{14}O_2$ was counted as barium carbonate, and the counts corrected for self absorption.

The activation of oxalate was followed by measuring the formation of hydroxamic acid in the presence of hydroxylamine. Hydroxamic acid was determined by adding ferric chloride, and measuring the absorbance at 540 mμ of the resulting colored complex (7). Hydroxylamine (2M, pH 7.5) was prepared just before use by mixing equal volumes of 4M KOH and 4M hydroxylamine hydrochloride.

Acetyl CoA and succinyl CoA were prepared by adding CoA to the corresponding anhydride (16) and assayed as the hydroxamate. Oxalate-1,2-C^{14} was obtained from the Radiochemical Centre, Amersham, and malonate-1,3-C^{14} from California Corporation for Biochemical Research. All cofactors were obtained from Sigma Chemical Company.

Table I

Cofactor Requirements for the decarboxylation of Oxalate-1,2-C^{14}

The complete reaction mixture contained 1 μmole oxalate-1,2-C^{14} (12,200 cpm); pH 6 supernatant material equivalent to 5 mg protein; 5 μmoles ATP; 0.1 μmole CoA; 5 μmoles $MgCl_2$; 0.1 μmole TPP; 5 μmoles glutathione; 100 μmoles potassium phosphate pH 7.2. Final volume 2.0 ml, pH 7.2. Incubation time, 30 minutes.

Components	$C^{14}O_2$ (cpm)
Complete	1,012
Minus ATP	8
" CoA	7
" $MgCl_2$	570
" Glutathione	888
" TPP	929
" P_i	1,255

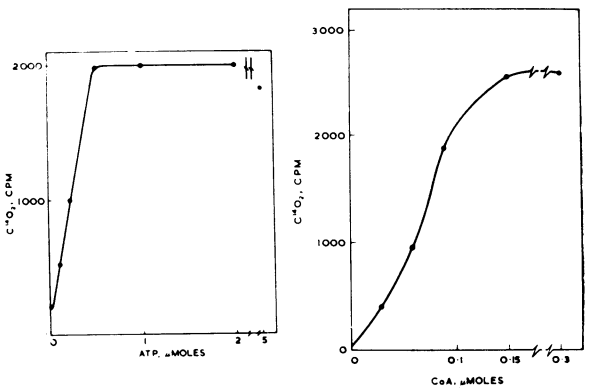


FIG. 1 (*left*). Effect of ATP concentration. Conditions were the same as those described for the complete reaction mixture in table I, except that each reaction mixture contained 1 μmole oxalate-1,2-C^{14} (29,000 cpm), a defatted extract of peas equivalent to 6.5 mg protein, and 100 μmoles tris chloride pH 7.2 was substituted for phosphate buffer. ATP was added as shown on the abscissa. Incubation time, 15 minutes.

FIG. 2 (*right*). Effect of CoA concentration. Conditions were the same as those described for the complete reaction mixture in table I except that each reaction mixture contained 1 μmole oxalate-1,2-C^{14} (29,000 cpm), a defatted extract of peas equivalent to 6.5 mg protein, and 100 μmoles tris chloride pH 7.2 was substituted for phosphate buffer. CoA was added as shown on the abscissa. Incubation time, 15 minutes.

Results

Cofactor Requirements. The results of a typical experiment, presented in table I, show that oxalate is readily decarboxylated by an extract prepared from cotyledons of germinating peas. Both ATP and CoA are required for the decarboxylation. Omission of $MgCl_2$ or glutathione caused a consistent decrease in the rate of decarboxylation. A slight (8%) decrease in the rate of decarboxylation was observed by omitting TPP[2]. It was possible to demonstrate a decrease of approximately 20% by omitting TPP when the supernatant enzyme was treated with the strongly basic resin Deacidite FF. Omission of P_i caused a small increase in the rate of decarboxylation.

The requirement for ATP is illustrated in figure 1. An apparent Michaelis constant (K_m) of the order of 10^{-4} M was obtained by determining the concentration of ATP required for one-half maximum activity. In some enzyme preparations a significant inhibition was obtained with concentrations of ATP greater than 2.5×10^{-3} M. This inhibition was relieved by the addition of a divalent cation, suggesting that the inhibition may be due to a complexing of divalent cations by excess ATP.

[2] Abbreviations: TPP, thiamin pyrophosphate; CTP, GTP, ITP and UTP, triphosphates of cytidine, guanosine, inosine and uridine, respectively.

Table II

Nucleotide Specificity for Decarboxylation of Oxalate

Each reaction mixture contained 1 μmole oxalate-1,2-C^{14} (58,400 cpm); defatted extract of peas equivalent to 1.61 mg protein; 5 μmoles glutathione; 0.1 μmole TPP; 5 μmoles $MgCl_2$; 0.2 μmole CoA; 100 μmoles tris chloride pH 7.2; 2 μmoles nucleotide, as shown below. Final volume 2.0 ml, pH 7.2. Incubation time, 30 minutes.

Nucleotide	$C^{14}O_2$ (cpm)
None	89
ATP	1,250
CTP	943
UTP	795
GTP	683
ITP	659
ADP	1,167
AMP	35

The activity of some nucleotides other than ATP is shown in table II. The triphosphates of cytidine, uridine, guanosine, and inosine all showed activity, which in each case was less than that observed with ATP. The activity observed with ADP was approximately equal to that observed with ATP. AMP was inactive. It is not known whether the nuclotides other than ATP were active per se, or by recycling catalytic quantities of adenosine phosphates. The relative activity observed in the presence of ATP, ADP, and AMP is consistent with the presence of adenylic kinase, which has been reported in defatted extracts of peas (8). Current studies on the properties of the oxalate-activating enzyme may provide more conclusive evidence on the nucleotide specificity.

The effect of CoA on the reaction is shown in figure 2. An apparent K_m of approximately 3.5×10^{-5} M is obtained by determining the concentration of CoA required for one-half maximum activity. The requirement for CoA is catalytic. Thus it could be shown in separate experiments that the addition of only 0.1 μmole of CoA catalyzed the decarboxylation of more than 1 μmole of oxalate.

The metal requirement was examined in more detail using a Chelex-treated enzyme (table III).

Table III

Requirement for a Divalent Cation

Conditions were the same as those described for the complete reaction mixture in table I, except for the use of crude or Chelex-treated enzyme, and the presence or absence of 2.5 μmoles of divalent cation. All divalent cations were added as the chloride salt. Incubation time, 30 minutes.

Enzyme	Metal added	Oxalate decarboxylated (μmoles/mg protein × 10^2)
Crude supernatant	None	1.9
Crude supernatant	Mg^{++}	3.3
Chelex-treated	None	0.2
Chelex-treated	Mg^{++}	2.9
Chelex-treated	Mn^{++}	3.7
Chelex-treated	Co^{++}	3.4

The crude supernatant fraction showed appreciable activity in the absence of Mg^{++}, addition of Mg^{++} increasing the specific activity approximately 1.7-fold. Treatment of the supernatant with Chelex reduced the activity in the absence of a divalent cation to a low level, and the addition of Mg^{++}, Mn^{++}, or Co^{++} restored full activity. Of the divalent cations tested, Mg^{++}, Mn^{++}, and Co^{++} were the most effective in restoring activity. Their relative activity, however, was not always as shown in table III. The reason for this apparent variation in the relative activity of these 3 divalent cations is not, at present, clear. The addition of Ca^{++}, Zn^{++}, or Fe^{++} gave a slight, but consistent stimulation.

The effect of Mg^{++} concentration on enzyme activity is shown in figure 3. Maximum stimulation was observed at a concentration of 10^{-3} M. Higher concentrations of Mg^{++} caused a decrease in the rate.

The stimulation caused by glutathione was not observed with other sulfhydryl compounds at the same concentration. Thus cysteine and 2,3-dimercaptopropanol had no effect. Thioglycolate inhibited the reaction approximately 40%. The reason for this apparent specificity of glutathione is not understood.

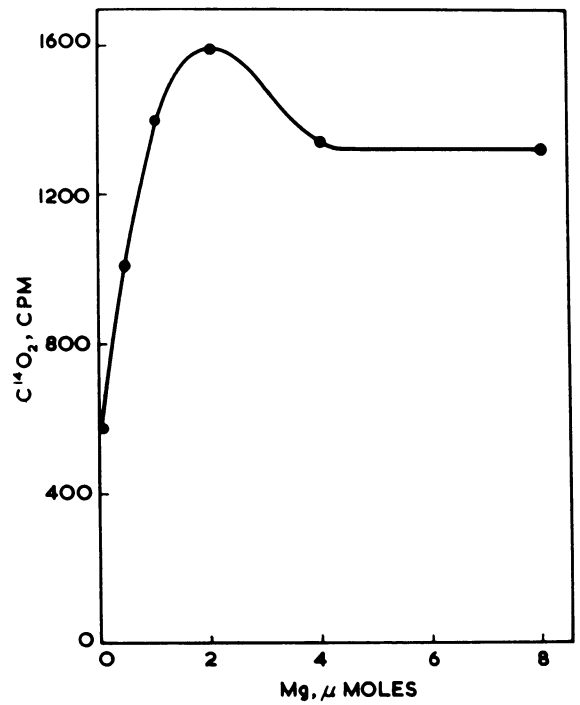
Activity as a Function of Enzyme Concentration. With a defatted extract the rate of $C^{14}O_2$ release was linearly proportional to the amount of enzyme added. With crude supernatant solutions prepared from cotyledons of germinating peas, on the other hand, a linear relationship could not be obtained consistently.

Other Properties. The pH optimum of the enzyme system is approximately 6.7 (fig 4). The rate of decarboxylation is not affected by substituting nitrogen for air in the gas phase. The enzyme system could be stored for 3 months at −15° without appreciable loss of activity. Repeated freezing and thawing, however, resulted in considerable loss of activity.

Malonate-1,3-C^{14} was completely inert in the system. The decarboxylation of oxalate is therefore not due to the activity of malonyl decarboxylase, which has been demonstrated in peanut mitochondria (2) and acetone powders of a number of plant tissues (5). Additional evidence for the specificity of the enzyme system for oxalate is presented below with studies of the oxalate-activating enzyme.

In addition to peas, the enzyme was demonstrated in lower activity in defatted extracts prepared from seeds of wheat, pumpkin, and bean, and from wheat germ. The preparation of these extracts was identical with that described for the preparation of a defatted extract of powdered pea seeds. Experiments to determine the activity of the enzyme system as a function of germination time were not definitive, since a linear relationship between activity and protein concentration could not consistently be obtained with crude supernatant preparations.

Stoichiometry of Oxalate Decarboxylation. The stoichiometry of the reaction was demonstrated by incubating 1.14 μmoles of oxalate-1,2-C^{14} (100,000 cpm) with crude supernatant enzyme under the conditions



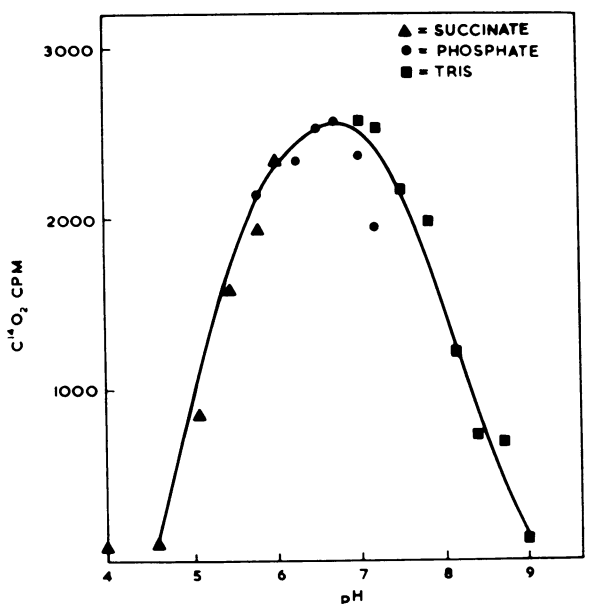


FIG. 3 (*upper*). Effect of Mg^{++} concentration. Conditions were the same as those described for the complete reaction mixture in table I, except that each reaction mixture contained Chelex-treated enzyme (containing 8.3 mg protein), and Mg^{++} concentration was varied as shown on the abscissa. Incubation time, 30 minutes.

FIG. 4 (*lower*). Effect of pH. Conditions were the same as those described for the complete reaction mixture in table I except that each reaction mixture contained 1 μmole oxalate-1,2-C^{14} (29,000 cpm); defatted extract of peas equivalent to 6.3 mg protein; 2 μmoles ATP; 100 μmoles succinate, phosphate or tris buffer at the pH shown on the abscissa. Incubation time, 15 minutes.

Table IV

Stoichiometry of Oxalate Decarboxylation

Each reaction mixture contained 1.14 μmoles oxalate-1,2-C^{14} (100,000 cpm); crude supernatant equivalent to 27 mg protein; 10 μmoles ATP; 0.1 μmoles CoA; 5 μmoles $MgCl_2$; 0.1 μmole TPP. Final volume 2.0 ml, pH 7.2. All samples were counted as barium carbonate after degradation as described in Materials and Methods. The apparent formation of 0.02 μmole of formate-C^{14} at zero time results from a degradation of up to 2% of oxalate-C^{14} by mercuric acetate.

Incubation time (min)	Oxalate-1,2-C^{14} decarboxylated (μmoles)	Formate-C^{14} formed (μmoles)	$C^{14}O_2$ formed (μmoles)
0	0.00	0.02	0.00
5	0.14	0.12	0.10
10	0.25	0.28	0.29
20	0.58	0.55	0.57
30	0.73	0.71	0.70
60	1.09	1.11	0.96
90	1.11	0.97	1.05

described in table IV. Chromatography in 95% ethanol: concentrated ammonia (100: 1 v/v) of ether extracts of reaction mixtures incubated for fixed times demonstrated that the decarboxylation of oxalate-C^{14} was accompanied by the formation of a radioactive compound migrating ahead of oxalate-C^{14} (3). This compound was identified as formate-C^{14} by A) 2 dimensional chromatography with authentic formate, using the ethanol: ammonia solvent described above in the first dimension (R_F, 0.37), and absolute ethanol: 16% aqueous ethylamine (4: 1 v/v) in the second dimension (R_F, 0.65); B) its steam volatility under acid conditions; C) degradation to $C^{14}O_2$ by mercuric acetate.

The stoichiometry of oxalate decarboxylation is illustrated by the results of table IV. Over a range of incubation times, the utilization of each mole of oxalate-C^{14} results in the formation of 1 mole of $C^{14}O_2$ and 1 mole of formate-C^{14}.

Evidence for a Direct Activation of Oxalate. In bacterial systems, oxalate has been demonstrated to be activated indirectly via a transferase reaction involving either acetyl CoA or succinyl CoA (13):

$$\left.\begin{matrix}\text{acetyl CoA or}\\ \text{succinyl CoA}\end{matrix}\right\} + \text{oxalate} \rightarrow \left.\begin{matrix}\text{acetate or}\\ \text{succinate}\end{matrix}\right\} + \text{oxalyl CoA}$$

The following observations suggest that the initial activation of oxalate in the enzyme system isolated from peas proceeds via a direct thiokinase reaction:

$$\text{oxalate} + \text{ATP} + \text{CoA} \rightarrow \text{oxalyl CoA} + \text{X}$$

A. No requirement for succinate or acetate could be demonstrated for the decarboxylation of oxalate. The rate of decarboxylation catalyzed by a Deacidite FF-treated supernatant fraction was not appreciably changed by the addition of acetate of succinate, when added separately or in combination.

B. Substitution of succinyl CoA or acetyl CoA for ATP and CoA reduces the rate of decarboxylation

Table V

Requirement of ATP, CoA, and Oxalate for Formation of Hydroxamic Acid

The complete reaction mixture contained 0–50% saturated ammonium sulfate fraction equivalent to 9.7 mg protein; 10 μmoles ATP; 0.2 μmole CoA; 20 μmoles potassium oxalate; 5 μmoles glutathione; 5 μmoles $MgCl_2$; 100 μmoles NaF; 400 μmoles hydroxylamine; 100 μmoles potassium phosphate pH 7.5. Final volume, 2.0 ml, pH 7.5. Incubation time, 30 minutes. The boiled enzyme was heated at 80° for 4 minutes.

Conditions	Absorbance 540 mμ
Complete	0.330
Complete (boiled)	0.030
Complete (stopped at zero time)	0.020
Minus ATP	0.030
Minus CoA	0.025
Minus oxalate	0.030

by 82% and 94% respectively. This observation indicates that, whereas some activation of oxalate may occur via a CoA transferase reaction, the major pathway involves a direct activation in the presence of ATP and CoA.

C. Addition of ammonium sulfate to defatted extracts of pea seeds yielded a fraction that catalyzed a rapid synthesis of hydroxamic acid. Omission of ATP, CoA, or oxalate markedly reduced the rate. Thus the results of table V show that, whereas the complete system was accompanied by the formation of hydroxamic acid equivalent to an absorbance at 540 mμ of 0.330, omission of ATP, CoA, or oxalate reduced this figure to less than 0.030. A low absorbance at 540 mμ was also observed with a reaction mixture containing boiled enzyme (absorbance of 0.030), or when the reaction mixture was stopped at zero time (absorbance of 0.020). The low rates observed in the absence of ATP, CoA, or oxalate are therefore probably closer to zero than indicated.

Of a number of substrates tested, oxalate was the most active (table VI). Low activity was observed in the absence of substrate or in the presence of malonate, acetate, or succinate. Rates obtained in the presence of glycolate or formate, although higher than those observed with malonate, acetate, or succinate, were still only of the order of 20% of the rate observed with oxalate. The mechanism of action, and further properties of this oxalate-activating enzyme are currently being investigated.

Table VI

Substrate Specificity of Oxalate-activating Enzyme

Each reaction mixture contained the compounds listed under table V, except for the presence or absence of 20 μmoles substrate, as shown below. All substrates were added as the potassium salt. Incubation time, 30 minutes.

Substrate	Absorbance 540 mμ
None	0.035
Oxalate	0.325
Malonate	0.045
Acetate	0.045
Succinate	0.040
Glycolate	0.060
Formate	0.065

Discussion

On the basis of the above observations, the scheme shown in figure 5 is suggested for the decarboxylation of oxalate. This reaction sequence is similar to that demonstrated in extracts of bacteria which utilize oxalate as a sole source of carbon (6, 13). In the bacterial system, however, it was demonstrated that oxalate is activated via a CoA transferase reaction involving either acetyl CoA or succinyl CoA. In the system from peas, the evidence strongly suggests that the initial activation of oxalate proceeds via a direct thiokinase reaction.

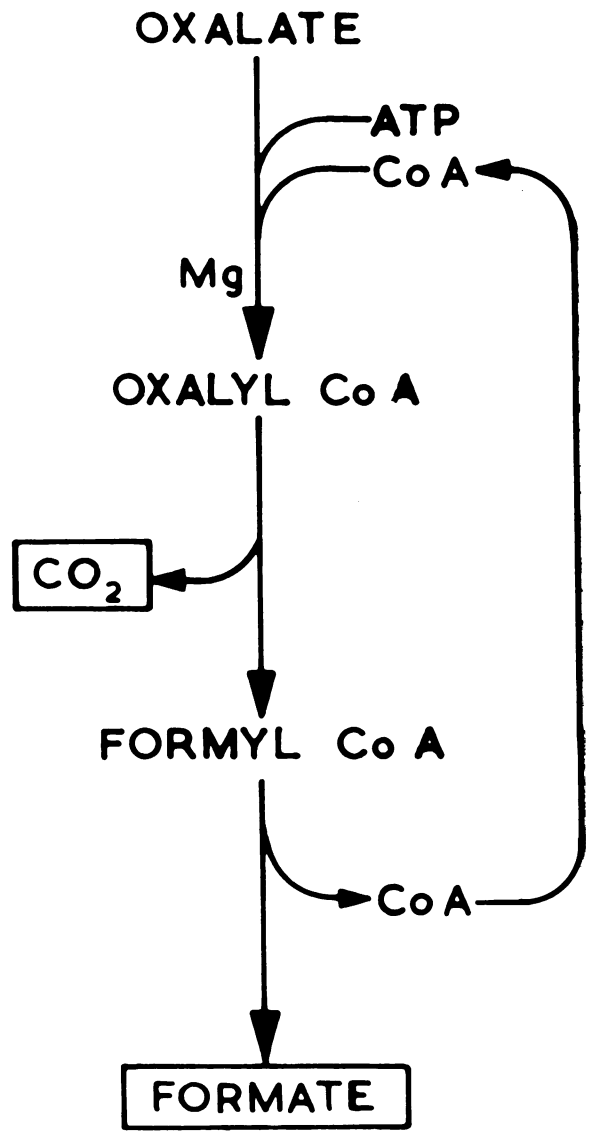


FIG. 5. Proposed scheme for decarboxylation of oxalate by extracts of peas.

Although it was observed that the requirement for CoA is catalytic, the mechanism whereby CoA is recycled is not understood. Two possible mechanisms are suggested. In the first, the high energy bond of formyl CoA is conserved, either by a transferase reaction:

$$\text{formyl CoA} + \text{oxalate} \rightarrow \text{oxalyl CoA} + \text{formate} \qquad \text{I}$$

or by a thiokinase reaction:

$$\text{formyl CoA} + \left.\begin{matrix}\text{AMP} + \text{pyrophosphate, or} \\ \text{ADP} + \text{orthophosphate}\end{matrix}\right\} \longleftrightarrow \text{formate} + \text{ATP} + \text{CoA} \qquad \text{II}$$

An enzyme catalyzing reaction I has been postulated to occur in extracts of bacteria utilizing oxalate as a sole source of carbon (13). The data presented under "Evidence for a direct activation of oxalate" argue against the initial activation of oxalate via acetyl CoA or succinyl CoA. These data do not, however, eliminate the possibility of a subsequent transfer of CoA from formyl CoA to oxalate (reaction I). To our knowledge, an enzyme catalyzing the direct activation of formate to formyl CoA (reaction II) has not been reported.

In the second suggested mechanism for recycling CoA, the high energy bond of formyl CoA is dissipated in the reaction:

$$\text{formyl CoA} + \text{water} \rightarrow \text{formate} + \text{CoA}$$

An enzyme catalyzing this reaction has been isolated from extracts of *Clostridium kluyveri* (14).

These 2 mechanisms could be differentiated by determining the stoichiometry of ATP in the decarboxylation of oxalate. The first mechanism, where the high energy bond of formyl CoA is conserved, requires only catalytic amounts of ATP. The second mechanism, where the high energy bond of formyl CoA is dissipated, requires 1 mole of ATP for each mole of oxalate decarboxylated. Unfortunately it was not practicable to determine the stoichiometry of ATP, since the preparations that catalyze the decarboxylation of oxalate also catalyze a rapid liberation of P_i from ATP.

At present it is difficult to suggest a physiological role of the enzyme system in plants. The participation of ATP and CoA suggests that the reaction may have some significance in the energetics of intermediary metabolism. In particular, the reaction sequence focuses attention on a possible role of formyl CoA in plant metabolism. The possibility was considered that formyl CoA may act as a source of 1 carbon units by way of a direct formylation of tetrahydrofolic acid to form formyl tetrahydrofolic acid. However, oxalate-1,2-C^{14}, when fed to slices of a number of plant tissues provided no evidence for this postulate. The predominant product of the metabolism of oxalate-1,2-C^{14} was $C^{14}O_2$, together with small amounts of formate-C^{14}. No label appeared in compounds which normally equilibrate with active 1 carbon units (e.g. serine, methionine) when oxalate-1,2-C^{14} was fed to slices of a number of plant tissues including pea cotyledons, carrots, parsnips, sweet potatoes, and swedes.

Summary

An enzyme system present in extracts of a number of plant tissues catalyzes an adenosine triphosphate- and coenzyme A-dependent decarboxylation of oxalate to formate. Glutathione, thiamin pyrophosphate, and divalent cations stimulate the reaction. Evidence is presented that the initial activation of oxalate proceeds directly via a thiokinase.

Acknowledgments

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Aspects of Nucleic Acid Composition in Gossypium [1]

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In a recent study Ergle and Katterman (10) compared the nucleotide compositions of DNA preparations isolated from genotypes of 8 stocks of American Upland cotton, *Gossypium hirsutum* L., and 1 stock of extra long stapled cotton, *G. barbadense*. While the over-all compositions of the 9 cottons did not differ from one another, the cytosine/5-methylcytosine ratios of 2 stocks differed significantly from those of the other varieties and races of hirsutum and the representative of barbadense.

Although the interspecific differences in the study just mentioned were insignificant, it seemed reasonable to expect that greater differences might be found among species of Gossypium in a larger and more genetically diverse sample. At least a survey of the species would form a sound basis for further work in this field. This paper reports the nucleotide compositions of DNA and RNA and the identity of the DNA-sugar component of 13 of the 23 recognized species of Gossypium.

[1] Received June 17, 1963.

Materials and Methods

Description of Species. Contemporary cotton botanists and geneticists now recognize 23 species (table I) of Gossypium; 4 of them are cultivated and

Table I

Species of the Genus Gossypium

Genome	Diploid species (n=13)	Geographic Origin
A_1	*G. herbaceum* L.	South Central Asia (Cultivated)
A_2	*G. arboreum* L.	South Central Asia (Cultivated)
B_1	*G. anomalum* Wawr. et Peyr.	South West and East Central Africa
B_2	*G. triphyllum* Hochr.	South West Africa
C_1	*G. sturtii* F. Muell.	South Central Australia
C_2	*G. robinsonii* F. Muell.	North West Australia
C_3	*G. australe* F. Muell.	North East Australia
D_1	*G. thurberi* Tod.	Southern Arizona and North West Mexico
D_2-1	*G. armourianum* Kearney	Islands in Gulf of Lower California
D_2-2	*G. harknessii* Brandg.	Islands and Coast of Gulf of Lower California
D_3-k	*G. klotzschianum* Anderss.	Galapagos Islands
D_3-d	*G. klotschianum* var. davidsonii (Kell.) H, S et St.	Coast of Gulf of Lower California
D_4	*G. aridum* (R and S) Skovsted	West Coast of Mexico
D_5	*G. raimondii* Ulb.	North Peru
D_6	*G. gossypioides* (Ulb.) Standley	South Central Mexico
D_7	*G. lobatum* Gentry	West Mexico
E_1	*G. stocksii* Mast.	East Arabia, West Pakistan
E_2	*G. somalense* (Gurke) H, S et St.	East Africa
E_3	*G. areysianum* (Deflers) H, S et St.	South Arabia
E_4	*G. incanum* (Schwartz) Hillcoat	South Arabia
E_5	*G. longicalyx* Hutch. et Lee	East Africa
	Tetraploid Species ($n=26$)	
$(AD)_1$	*G. hirsutum* L.	Southern Mexico and Central America (Cultivated)
$(AD)_2$	*G. barbadense* L.	South America (Cultivated)
$(AD)_3$	*G. tomentosum* Nutt.	Hawaiian Islands