Malate synthetase in germinating seeds

The enzymic condensation of glyoxalate with acetylCoA to yield malate (malate synthetase reaction) has been demonstrated in acetate-grown *Escherichia coli*[1] and *Pseudomonas fluorescens*[2]. Studies involving the incorporation of [^{14}C]acetate into malate have implicated this reaction in the process of conversion of fat to carbohydrate in germinating high-fat seeds[3,4]. It is the purpose of this note to demonstrate conclusively the malate synthetase reaction in peanut extracts and further to support its participation in the process of fat conversion by a distribution study in various germinated seeds. Data will also be presented on the substrate specificity of the enzyme.

When an extract of the cotyledons of peanuts germinated for 5 days was incubated with glyoxalate and an acetylCoA-generating system (acetyl phosphate, CoA and transactylase) there was a disappearance of glyoxalate and acetyl phosphate with concomitant accumulation of malate. As shown in Table I, omission of either the enzymic or substrate components results in the absence of the reaction. The requirement for glutathione is solely for the generation of acetylCoA since subsequent experiments with acetylCoA (Fig. 1) showed no such requirement. Malate was further indentified as the product of the reaction by chromatography in butanol saturated with HCOOH.

TABLE I

MALATE SYNTHETASE OF PEANUT EXTRACT

In Expt. 1, the complete system contained 6.5 μmoles glyoxalate, 7.5 μmoles acetyl phosphate, 150 μmoles Tris buffer, pH 7.5, 50 μmoles KCl, 0.08 μmole CoA, 5 μmoles $MgCl_2$, 2.5 μmoles glutathione, 5–10 units transacetylase and 0.06 ml peanut supernatant (5-day-germinated peanuts blended for 1 min with 1.5 vol. of 0.1 *M* Tris, pH 8.0, filtered through cheese-cloth and centrifuged for 15 min at 12,000 × *g*), in a final vol. of 1.2 ml. Two sets of vessels were incubated for 20 min at 30°. One set was then deproteinized with trichloroacetic acid and glyoxalate was determined as the 2,4-dinitrophenylhydrazone[6]. The second set was deproteinized by heating for 2 min at 100° and malate was determined with malic dehydrogenase. The method involves addition of an aliquot containing less than 0.15 μmole malate to 2.0 ml glycine–semicarbazide (0.2 *N* glycine containing 10 g semicarbazide·HCl/100 ml adjusted to pH 10.4 with NaOH), and 0.5 μmole DPN in a vol. of 3.0 ml. The absorbance at 340 mμ is noted and 0.03 ml of an $(NH_4)_2SO_4$ suspension of malic dehydrogenase (Worthington Biochemical) is added. The change in absorbance at 340 mμ is again noted after 6 h. Under these conditions the increase in absorbance is proportional to malate up to 0.15 μmole with an absorbance change of 0.128 for 0.1 μmole. With these concentrations there is no further change in absorbance at 340 mμ after 6 h. Expt. 2 was similar to Expt. 1 except for 9.6 μmoles glyoxalate, 5.0 μmoles acetyl phosphate and 150 μmoles of Tris buffer, pH 8.0, and 0.05 ml of peanut supernatant. Acetyl phosphate was determined by the hydroxamate reaction[7]. The results are corrected for non-enzymic glyoxalate disappearance and acetyl phosphate hydrolysis by acetyl phosphatase in the peanut extract.

Conditions	*Expt. 1*		*Expt. 2*
	Glyoxalate disappeared (μmoles)	*Malate formed (μmoles)*	*Acetyl phosphate disappeared (μmoles)*
Complete system	1.8	2.2	1.6
No peanut enzyme	0.0	0.0	0.0
No transacetylase	0.1	0.1	0.0
No CoA	0.2	0.1	0.1
No $MgCl_2$	—	—	1.4
No glutathione	0.2	—	0.2
No glyoxalate	—	0.2	0.0
No acetyl phosphate	0.1	0.1	—

Abbreviations: CoA, coenzyme A; Tris, tris(hydroxymethyl)aminomethane.

Examination of extracts of the cotyledons of germinated castor beans and soybeans showed that only 10 to 20 % of the activity could be extracted into the supernatant by blending in either phosphate or Tris buffer. Accordingly, a distribution study was performed with whole homogenates of various tissues. The results (Table II), showing that the enzyme is present in large amounts only in high fat seeds, lend support for the function of malate synthetase in the utilization of fat. The enzyme is

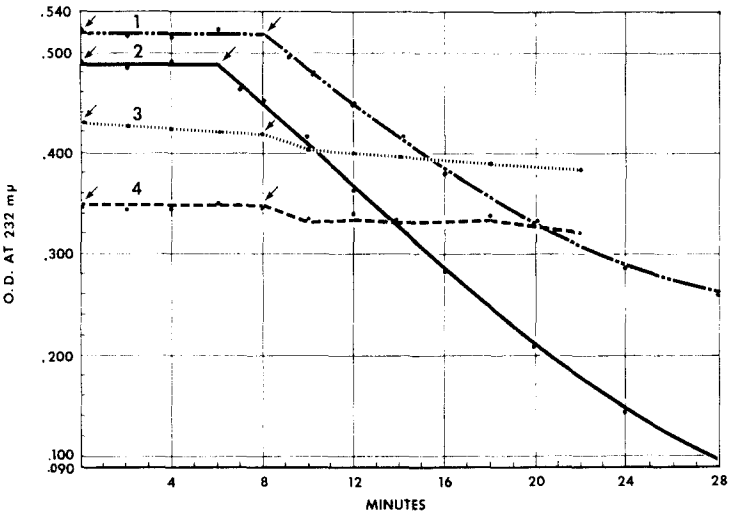

Fig. 1. All experimental cuvettes contained 100 μmoles Tris buffer, pH 8.0, 5 μmoles $MgCl_2$. acyl thiolester and water to 1.0 ml. Cuvettes 1 and 2 contained, respectively, 0.1 and 0.2 μmole acetylCoA. Cuvettes 3 and 4 contained, respectively, 0.1 μmole acetyl glutathione and acetyl pantetheine. The blank cuvette contained sufficient acetyl pantetheine to bring the experimental cuvette to the desired absorbance. At the first arrow in cuvettes, 1, 3, and 4, 0.02 ml of 6-day-peanut supernatant (diluted 10-fold with water) was added and at the second arrow 0.01 ml 0.12 *M* potassium glyoxalate was added. In cuvette 2, the order of addition was reversed. All values are corrected for absorbance due to the component added. All acyl thiolesters were prepared by the method of SIMON AND SHEMIN[8].

TABLE II

DISTRIBUTION OF MALATE SYNTHETASE

The assay system was that described in Table I, Expt. 1. Under these conditions glyoxalate disappearance is proportional to enzyme concentration up to 1.6 μmoles removed. The chicken-liver and pig-heart preparations were 10% extracts of acetone powders in 0.1 *M* Tris, pH 8.0. All other preparations were homogenates in 1.5 vol. 0.1 *M* Tris, pH 8.0, filtered through cheese-cloth. The seeds were germinated for 5 days on wet towels at appropriate temperatures. The sunflower roots were obtained from a 5-day seedling and the leaves from a young field plant. Barley embryos were tested after overnight standing on moist filter paper. At least 3 points within the range of linearity were obtained for each sample.

Material	*μmoles/ml/h*	*Material*	*μmoles/ml/h*
Sunflower seed	156	Barley seed	0
Cotton seed	144	Barley embryo	0
Peanut seed	91	Kidney bean seed	0
Castor bean seed	84	Sunflower root	1.2
Soybean seed	48	Peanut leaves	0
Pea seed	14.1	Chicken-liver extract	0
Corn seed	6.0	Pig-heart extract	0

apparently restricted to the cotyledon as several analyses of leaf and root homogenates showed no activity. In experiments not shown here, it was found that the enzyme is present in extracts of acetone powders of eithers peanut or castor-bean cotyledons.

In order to test the specificity of the malate synthetase reaction, various compounds were compared with acetyl phosphate in the assay system of Table II. With acetyl pantetheine, acetyl glutathione and fluoroacetyl phosphate[5], 0.1, 0.4, and 0.1 μmoles glyoxalate disappeared. The data with fluoroacetyl phosphate and acetyl pantetheine are within experimental error while the apparent activity with acetyl glutathione may have been due to liberation of free thiol by a thiolesterase in the extract, with subsequent non-enzymic condensation with glyoxalate. To test this point, the reaction was followed by disappearance of thiol ester at 232 mμ. As shown in Fig. 1 neither acetyl pantetheine nor acetyl glutathione are reactive in the malate synthetase reaction and the extract does contain acetyl glutathione thiolesterase. These experiments further establish that acetylCoA is the reactive component and that both glyoxalate and enzyme are required for the reaction. In similar spectrophotometric experiments it was found that both propionylCoA and butyrylCoA are not active, indicating complete specificity of the reaction for acetylCoA.

Two types of experiments were performed to test the reversibility of the reaction (a) incubation of malate, CoA, and enzyme with hydroxylamine and analysis for acethydroxamic acid (b) incubation of malate, CoA and enzyme with a system of removal of acetylCoA (transacetylase and arsenate) and analysis for glyoxalate. The negative results obtained in both experiments indicate that the equilibrium is far in the direction of malate and that detection of reversibility will require an extremely sensitive assay.

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A method for plotting ultracentrifuge diagrams for polydisperse systems

The results of ultracentrifugal analyses, as generally recorded photographically, show a plot of the rate of change of refractive index (dn/dx) *versus* the distance from the center of rotation (x). To show changes occurring with time, several pictures are necessary. We therefore decided to examine the alternate possibility of using a plot of dn/dx *versus* time at one value of x since the results of one ultracentrifugal analysis may thus be presented in one picture. This is of some advantage where, by the usual