MALONATE-INHIBITION OF ALLOSTERIC PHOSPHOENOLPYRUVATE CARBOXYLASE FROM SETARIA ITALICA*

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SUMMARY: Malonate inhibited phosphoenolpyruvate carboxylase activity in crude extracts of Setaria italica Beauv. and Pennisetum typhoides S & H leaves. Kinetic studies revealed the competetive inhibition of partially purified phosphoenolpyruvate carboxylase preparation from Setaria italica. Glucose-6-phosphate activated the enzyme. Oxalacetate also competetively inhibited the enzyme and malate in a noncompetetive manner. Results clearly indicated the allosteric nature of phosphoenolpyruvate carboxylase from Setaria italica.

Bassham et al. (1) reported as early as in 1950 that malonate inhibited the labelling of malate during photosynthesis by Chlorella. Although the mechanism of action was not known, they assumed that it possibly acted via the inhibition of Krebs cycle. The interest in malonate was revived when it was shown to inhibit totally $^{14}CO_2$ incorporation by leaves of sugarcane (2). The results with sugarcane leaves were in contrast to those of Bassham et al. (1) where $^{14}CO_2$ incorporation through the Calvin cycle was not affected. Malonate was also known to block the formation of malate in succulent (CAM)** plants (3). The common feature of malate formation in C_4 plants (such as

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** Abbreviations used were: RuDP, Ribulose 1,5-diphosphate; Chl, Chlorophyll; C_4 plants, Plants possessing C_4 dicarboxylic acid pathway of photosynthesis; CAM, Crassulacean acid metabolism; PEP, Phosphoenolpyruvate.

sugarcane) and CAM plants is due to the predominance of PEP carboxylation over RuDP carboxylation (4). High PEP carboxylase activity is a characteristic feature of C_4 plants (5). In view of its classical role as a competetive inhibitor of succinic dehydrogenase (6), we examined the effect of malonate on PEP carboxylase prepared from Setaria italica, a C_4 plant possessing the C_4 dicarboxylic acid pathway of photosynthesis (7). Since the data on PEP carboxylase from C_4 plants is still limited, we also studied the properties of the enzyme in more detail.

MATERIALS AND METHODS

Plants of Setaria italica Beauv. var. H-1 and Pennisetum typhoides S & H var. AKP-2 were grown in seed pans under 12-hour photoperiod for three weeks. The leaves were harvested and ground in four volumes of 50 mM Tris-HCl buffer, pH 7.8 containing 5 mM dithiothreitol; 1 mM EDTA; 2 mM $MgCl_2$ and 2-mercaptoethanol. The extract was filtered through four layers of cheese cloth. An aliquot was set aside for chlorophyll by the method of Arnon (8). The extracts were assayed for PEP carboxylase activity by assaying PEP dependent radioactive HCO_3^- incorporation (7).

PEP carboxylase was initially extracted into 0.15 mM Tris-HCl buffer pH 7.8 containing 150 mM 2-mercaptoethanol and 1.5 % polivinylpyrrolidone (molecular weight 10,000). The enzyme was partially purified by ammonium sulfate fractionation and elution from Sephadex G-25 column (7), and assayed as above.

Other details are described in the text.

RESULTS AND DISCUSSION

Malonate inhibited PEP carboxylase activity in crude extracts of both Setaria italica and Pennisetum typhoides (Fig.1).

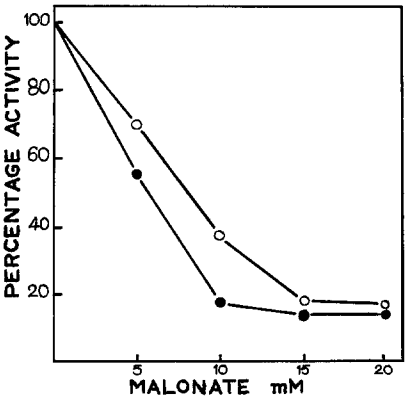

Figure 1. Inhibition of PEP carboxylase activity by malonate in crude extracts of Setaria italica and Pennisetum typhoides leaves.

O—O Setaria italica; ●——● Pennisetum typhoides. The activities in control sets were 748 and 1078 μ moles/mg chl/hr for Setaria italica and Pennisetum typhoides respectively. The reaction mixture (2 ml) contained 50 mM Tris-HCl buffer pH 8.0; 2 mM $MgCl_2$; 2 mM $NaH^{14}CO_3$ (1.6 mCi/m mole); 1.5 mM PEP and the enzyme. The reaction was stopped after 3 min at 30°C with an equal volume of 1 N HCl saturated with 2,4-dinitrophenylhydrazine. An aliquot was examined for incorporated radioactivity.

This explains not only the failure of labelling of malate in Chlorella and CAM plants in the presence of malonate, but also the blocking by malonate of $^{14}CO_2$ incorporation by sugarcane leaves. The differential effect of malonate on normal $^{14}CO_2$ incorporation by Chlorella and sugarcane is reasonable. PEP carboxylation is the main route of CO_2 fixation by sugarcane leaves (9), but the principal entry for CO_2 in Chlorella is

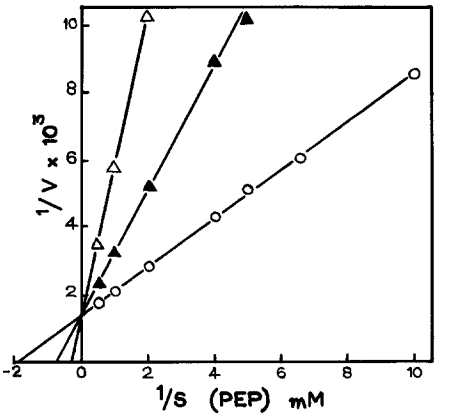

Figure 2. Competetive inhibition of partially purified PEP carboxylase from Setaria italica leaves by malonate.

O——O Control; ▲——▲ 5 mM malonate; Δ——Δ 10 mM malonate. The preparation exhibited an activity of 712 μ moles/mg protein/hr. The reaction mixture for enzyme assay was same as described for Figure 1.

RuDP carboxylase of Calvin cycle (10) and malate is only a secondary product of photosynthesis.

Studies of the inhibition by malonate indicated that this was competetive with PEP (Fig.2). Ki (malonate) was 2.15 mM. The observed Km for PEP in the control without inhibitor (0.53mM) is in agreement with the values observed previously (7,12). Malonate is well known to be a classical example of competetive inhibitor of succinic dehydrogenase (6). The present observations readily explain the inhibitory effect of malonate on $^{14}CO_2$ incorporation by mesophyll chloroplasts of P. typhoides and S. italica (7) and sugarcane chloroplasts (11).

Of the various metabolites tested glucose-6-phosphate was found to stimulate the enzyme activity (Table I). Oxalacetate and malate decreased the activity significantly whereas citrate

TABLE I

EFFECT OF VARIOUS METABOLITES ON PEP CARBOXYLASE FROM SETARIA ITALICA*

Compound	Percentage Activity**	Compound	Percentage Activity**
Oxalacetate	46	Glucose-1-phosphate	107
Malate	52	Glucose-6-phosphate	168
Pyruvate	87	Ribulose 1,5-diphosphate	98
Citrate	83	Ribose-5-phosphate	103
Aspartate	94	3-phosphoglycerate	95
Succinate	92	Fructose 1,6-diphosphate	102

* All the compounds were tested at 5 mM concentration. Assay conditions were as at Fig. 2.

** Over control (without any added metabolite).

and pyruvate were slightly inhibitory. The stimulating effect of glucose-6-phosphate confirms earlier reports (12,13) and emphasizes the possible regulatory role of this compound on the carboxylation of PEP in leaves of C_4 plants. Oxalacetate and malate were already known to be effective inhibitors of PEP carboxylases in plants (13,14,15). Detailed investigation on the effect of oxalacetate and malate indicated clearly that the former is a competetive inhibitor whereas the latter inhibits in a noncompetetive way (data not shown). The Ki for oxalacetate was calculated to be about 1 mM and Ki (malate) was 5 mM.

Previous reports have suggested that the photosynthetic PEP carboxylase is subjected to allosteric regulation (12,13).

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