GLYCINE ACTIVATION OF PEP CARBOXYLASE FROM MONOCOTYLEDONEOUS C_4 PLANTS*

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SUMMARY : Phosphoenolpyruvate carboxylase from Zea mays leaves was found to be activated by L-glycine and inhibited by maleic acid, but was not affected by the effectors for the bacterial enzymes. The activating effect of L-glycine was observed with all the enzymes from leaves of several monocotyledoneous C_4 plants, while the enzymes from dicotyledoneous C_4 plants and mono- and dicotyledoneous C_3 plants were not activated by L-glycine. Maleic acid inhibited the enzyme activities of all the higher plants tested.

PEP** carboxylase (EC 4.1.1.31) is widely distributed in higher plants and bacteria. The enzyme from Enterobacteriaceae has been found to be an allosteric enzyme which is activated by acetyl-CoA (1) and fructose 1,6-diphosphate (2,3) and inhibited by L-aspartate, L-malate and fumarate (4,5). In C_4 plants, PEP carboxylase catalyses the first reaction of a pathway termed C_4 dicarboxylic acid pathway of photosynthesis (6). It is of interest to examine whether the activities of C_4 plants enzymes are also subject to metabolic regulation as in Enterobacteriaceae and whether the enzymes from C_4 and C_3 plants are controlled differently. In C_4 plants, recently, PEP carboxylase from maize leaves was inhibited by oxaloacetate (7) and the enzyme from leaves of Pennisetum purpureum was activated by intermediates of

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** Abbreviation : PEP, phosphoenolpyruvate; C_4 plants, the plants having both C_4 dicarboxylic acid pathway and reductive pentose phosphate pathway in photosynthesis; C_3 plants, the plants having only reductive pentose phosphate pathway in photosynthesis.

the photosynthetic carbon reduction cycle such as glucose-6-phosphate and fructose-6-phosphate and inhibited by phosphoglyceric acid (8). In C_3 plants, the enzyme from cotton leaf tissue was inhibited by some organic acids and nucleotides (9).

This communication reports the activation of PEP carboxylase by L-glycine and the inhibition by maleic acid in several monocotyledoneous C_4 plants. Comparative studies of the enzymes from C_4 and C_3 plants are also described.

MATERIALS AND METHODS

Zea mays, Saccharum officinarum, Sorghum bicolor, Avena sativa, Triticum aestivum, Hordeum vulgare and Nicotiana tabacum were grown in a green house. Miscanthus sinensis, Oryza sativa, Amaranthus retroflexus, Amaranthus tricolor and Phytolacca americana were obtained from a field during summer and autumn in Urawa, Japan. Spinacia oleracea was from a market. Plant leaves were harvested usually in the afternoon at a fine day.

10 g of leaf tissue were homogenized in a motar and pestle at 4 °C with an equal weight of quartz sand and 20-30 ml of 100 mM Tris-HCl buffer (pH 8.5) containing 14 mM 2-mercaptoethanol. The resulting homogenate was passed through four layers of cheese cloth and the filtrate was centrifuged at 28,500 x g for 10 min. PEP carboxylase in the supernatant was fractionated by 30-60 % saturation of ammonium sulfate. The activity of PEP carboxylase was measured spectrophotometrically at 340 mμ with a double-beam Shimazu Double-40 R recording spectrophotometer by coupling with a pig heart malate dehydrogenase (EC 1.1.1.37). The standard reaction mixture contained 1 mM PEP, 10 mM $MgSO_4$, 0.1 mM NADH, pig heart malate dehydrogenase (3.6 international units), and the enzyme in a total volume of 2.0 ml. The reaction was carried

TABLE I

Effect of Various Metabolites on the PEP Carboxylase from Zea mays leaves.

Addition (mM)		Relative Activity
None	(5)	100
L-Aspartate	(5)	100
Fumarate	(5)	89.0
L-Malate	(5)	86.8
FDP	(5)	84.0
Acetyl-CoA	(0.2)	103
Maleate	(5)	20.0
L-Glycine	(5)	170
L-Alanine	(5)	115
L-Serine	(5)	108
Glycolate	(5)	97.0
Succinate	(5)	82.0
Citrate	(5)	89.0
α-Ketoglutarate	(5)	102
Pyruvate	(5)	96.0
ATP	(5)	50.0
ADP	(5)	75.0
$NADP^+$	(5)	101

The activities are expressed relative to control (None = 100). For each experiment 3.5 units of the enzyme (correspond to 11.2 mμmoles of NADH oxidized per min) was used. Assay conditions are the same as described in MATERIALS AND METHODS except for the addition of various metabolites on the reaction mixture.

out at 30 °C and initial rates were used in calculation of all activities. One unit of the enzyme was defined as the amount causing an absorption change of 0.01 at 340mμ per min under these assay conditions.

RESULTS AND DISCUSSIONS

Various metabolites were surveyed for the effect on the

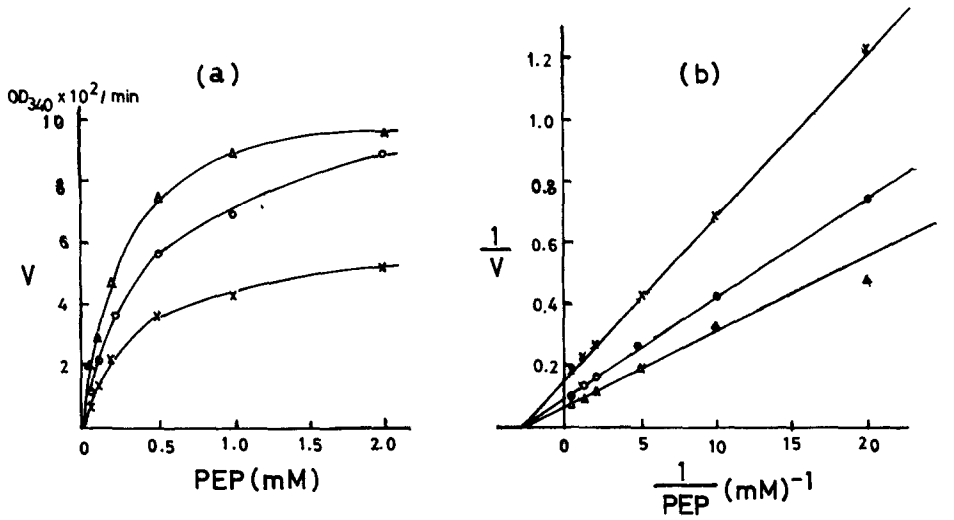

Fig. 1 Effects of L-glycine and maleic acid on Zea mays PEP carboxylase with various concentrations of PEP. (a) Initial velocity versus PEP concentration. (b) Double-reciprocal plots.

Experimental conditions are the same as those described in Table I except for the concentrations of PEP and maleic acid. 7.0 units of the enzyme was used. —o—o—, control ; —Δ—Δ—, 5 mM L-glycine ; —x—x—, 1 mM maleic acid.

activity of PEP carboxylase from Zea mays. As shown in Table 1, 0.2 mM acetyl-CoA and 5 mM FDP, activators of bacterial enzymes, did not show any significant effect on the activity. These results were similar to those reported with PEP carboxylase from maize leaf lamina (7) and cotton leaf tissue (9). However, Zea mays enzyme was significantly activated by 5 mM of L-glycine and inhibited by 5 mM of maleic acid. These substances did not affect the PEP carboxylase activity of Esherichia coli (6). The regulation of Zea mays PEP carboxylase, therefore, may be different from that of bacteria. The activation of Zea mays enzyme by L-glycine seems to be specific, because other amino acids and nucleotides had little or no effect. Inhibition by ATP and citrate were released by the addition of Mg^{2+}, suggesting these effects by chelation.

In order to investigate the nature of the activation by L-

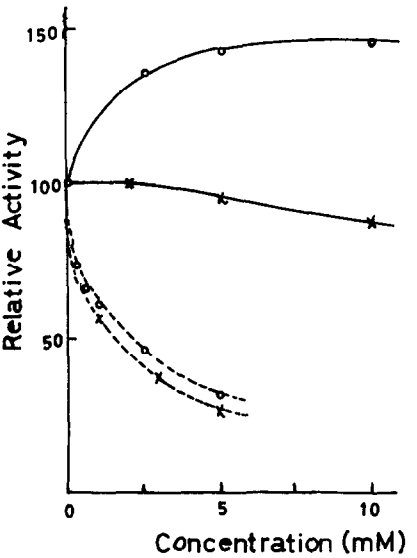

Fig. 2 Effects of increasing concentrations of L-glycine and maleic acid on PEP carboxylase from Zea mays and Spinacia oleracea.

Experimental conditions are the same as described in Table I except for the concentrations of L-glycine and maleic acid. 3.5 units of Zea mays enzyme and 2.5 units of Spinacia oleracea enzyme were used for each experiment. —o——o—, Zea mays (L-glycine) ; —x——x—, Spinacia oleracea (L-glycine);---o-----o--- , Zea mays (maleic acid) ; ---x----x---, Spinacia oleracea (maleic acid).

glycine and the inhibition by maleic acid, effects of these compounds on PEP saturation curve were studied with Zea mays enzyme. As shown in Fig. 1-(a), PEP saturation curve showed a normal hyperbola, regardless of the presence or absence of L-glycine and maleic acid. From the double reciprocal plots of the reaction velocity against substrate concentration, as shown in Fig. 1-(b), Km (PEP) was 0.31 mM and this was not affected by L-glycine and maleic acid. Maleic acid showed a typical non-competitive inhibition with an inhibitor constant (Ki) of 1.8 mM.

As a first step to examine whether the effects of L-glycine and maleic acid are ubiquitous for the plant PEP carboxylases, responces of the enzymes from Zea mays (C_4 plant) and Spinacia

TABLE II

Effects of L-Glycine and Maleic Acid on the Activity of PEP Carboxylase from Various Higher Plants

Species	Relative Activity		
	Control	L-Glycine (5 mM)	Maleic acid (5 mM)
C_4-Monocotyledoneae			
Zea mays	100	161	25.5
Saccharum officinarum	100	139	17.7
Miscanthus sinensis	100	164	26.0
Sorghum bicolor	100	183	35.2
C_4-Dicotyledoneae			
Amaranthus retroflexus	100	108	26.0
Amaranthus tricolor	100	103	42.7
Portulaca oleracea	100	90.8	33.2
C_3-Monocotyledoneae			
Oryza sativa	100	102	19.5
Avena sativa	100	108	25.7
Triticum aestivum	100	94.7	21.3
Hordeum vulgare	100	90.0	35.2
C_3-Dicotyledoneae			
Spinacia oleracea	100	94.5	20.8
Nicotiana tabacum	100	88.1	0.0
Phytolacca americana	100	100	8.3

Experimental conditions are the same as those described in Table I except for the unit of the enzyme (1-10 units of the enzyme was used for each experiment).

oleracea (C_3 plant) were compared by changing the concentration of these compounds. As shown in Fig. 2, *Zea mays* enzyme was activated by 2.5-10 mM of L-glycine, but the enzyme from *Spinacia oleracea* was rather inhibited by 5.0-10 mM of L-glycine. Maleic acid showed a strong inhibition for both enzymes. These data indicate at least two possibilities to be considered : (1) Glycine

activation is a special property of Zea mays enzyme. (2) a common property of all the C_4 plants enzymes. Therefore, the effect of L-glycine and maleic acid were surveyed for the enzyme extracts obtained from a variety of plant leaves including C_4 and C_3 species. As shown in Table II, all the enzymes from monocotyledoneous C_4 plants including Zea mays, Saccharum officinarum, Miscanthus sinensis and Sorghum bicolor were activated 40-80 % by 5 mM of L-glycine. However, the enzymes from dicotyledoneous C_4 plants such as Amaranthus retroflexus, Amaranthus tricolor and Portulaca oleracea were not activated by L-glycine. PEP carboxylase was detected from all the C_3 plants indicated in Table II, although activities were low in comparison to C_4 plants. All the enzymes from C_3 plants were not affected by L-glycine, irrespective of monocotyledons (Oryza sativa, Avena sativa, Triticum aestivum and Hordeum vulgare) and dicotyledons (Spinacia oleracea, Nicotiana tabacum and Phytolacca americana). From these results, the following conclusion might be proposed that the activating effect of L-glycine on PEP carboxylase is a common property of all the monocotyledoneous C_4 plants.

On the other hand, maleic acid inhibited all the plants enzymes tested regardless of C_4 or C_3 and monocotyledons or dicotyledons. It was of interest that a bacterial PEP carboxylase was not significantly affected by maleic acid (5).

Detailed mechanisms of the activation by L-glycine and the inhibition by maleic acid and physiological significances of these phenomena are currently under investigation in this laboratory.

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