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On the Molecular Mechanism of Maize Phosphoenolpyruvate Carboxylase Activation by Thiol Compounds[1]

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ABSTRACT

Incubation of purified phosphoenolpyruvate carboxylase from *Zea mays* L. leaves with dithiothreitol resulted in an almost 2-fold increase in the enzymic activity. The activated enzyme showed the same affinity for its substrates and the same sensitivity with respect to malate and oxalacetate inhibition. The activation induced by dithiothreitol was reversed by diamide, an oxidant of vicinal dithiols, suggesting that the redox state of disulfide bonds of the enzyme may be important in the expression of the maximal catalytic activity.

Titration of thiol groups before and after activation of maize phosphoenolpyruvate carboxylase by dithiothreitol shows an increase of the accessible groups from 8 to 12 suggesting that the reduction of two disulfide bonds accompanied the activation. The thiols exposed by the treatment with dithiothreitol were available to reagents in nondenatured enzyme and two of them were reoxidized to a disulfide bond by diamide. It is concluded that the mechanism of phosphoenolpyruvate carboxylase activation by dithiothreitol involves the net reduction of two disulfide bonds in the enzyme.

Phosphoenolpyruvate carboxylase catalyzes the reaction of PEP[3] with HCO_3^- to form oxalacetate. The enzyme is widely distributed among a variety of organisms. In C_4 and Crassulacean plants, the carboxylase catalyzes the primary carbon fixation for the photosynthetic pathway in mesophyll cells, whereas in bacteria, algae, and C_3 plants, it plays an auxiliary role (16, 18).

PEP carboxylase requires a divalent metal ion for activity, but the role in catalysis is still a matter of some controversy (15, 16), and uses HCO_3^- rather than CO_2 as substrate (3, 17). In C_4 plants, PEP carboxylase is allosterically activated by glucose-6-P (2) and is inhibited by some organic acids such as oxalacetate and malate, which are the primary products of carboxylation *in vivo* (5, 10, 20, 21, 28).

Evidence that thiol groups play a role in the activity of PEP carboxylase from C_4 plants has been previously reported (1, 4, 14, 25, 26). Recently, Manetas and Gavalas suggested that the activity of PEP carboxylase from C_4 (12) and Crassulacean (13) plants might be regulated *in vivo* by a reversible redox change of sulfhydryl groups. Moreover, incubation of partially purified PEP carboxylase from maize (4) and *Amaranthus viridis* (22) with DTT resulted in an almost 2-fold increase in the enzyme activity while *o*-iodosobenzoate, an oxidant of vicinal dithiols, induced a total inactivation of the enzyme (22) pointing out the role of dithiols in the activity of the enzyme.

In the present work, we have examined the mechanism of activation of PEP carboxylase purified from maize leaves by incubation of the enzyme with DTT at room temperature. The effects of substrates and modulators on the DTT-activated enzyme as well as the content of free and accessible thiol groups of the enzyme are described.

MATERIALS AND METHODS

Materials. PEP (monopotassium salt), DTT, diamide, iodoacetamide, iodoacetic acid (sodium salt), NADH, cis-oxalacetic acid, porcine heart malic dehydrogenase, and *N*-ethylmaleimide were obtained from Sigma Chemical Co.

The *N*-[ethyl-2-^{3}H]maleimide (300 Ci/mol) was purchased from New England Nuclear, 2,5-diphenyloxazole and naphthalene were from Beckman, and 1,4-dioxane was from Baker Chemical Co. All other reagents were of analytical grade.

Enzyme Purification and Assay. PEP carboxylase was extracted from leaves harvested from *Zea mays* L. (Simple hybrid A_x 252) of about 1 month of age. The enzyme was purified according to O'Leary *et al.* (17) except that Sepharose 6B and sucrose density gradient centrifugation were done instead of Sephacryl S-200 and hydroxylapatite chromatography steps (7).

The fractions containing PEP carboxylase activity recovered from the DEAE-cellulose column were precipitated with 60% saturated $(NH_4)_2SO_4$, centrifuged at 15,000*g* for 15 min, and redissolved in a minimal volume of buffer containing 50 mM Tris-HCl (pH 7.3), 0.1 mM EDTA, 10% glycerol, and 10 mM β-mercaptoethanol. This concentrated enzyme was chromatographed on a 2- × 80-cm Sepharose 6B column that had been equilibrated with the same buffer. Two-ml fractions were collected and those with PEP carboxylase activity were pooled and precipitated with $(NH_4)_2SO_4$ (60% saturation).

After centrifugation at 15,000*g* for 15 min, the pellet was redissolved in a small volume of 50 mM Tris-HCl (pH 7.3), 1 mM EDTA, and 10 mM β-mercaptoethanol and desalted by Sephadex G-50 column chromatography. Then the protein (5 mg/ml) was loaded on a linear sucrose gradient (10–30%) prepared in the same buffer. After centrifugation at 180,000*g* for 135 min at 25°C in a Sorvall OTD-65 ultracentrifuge, the band of PEP carboxylase activity was collected and the sucrose eliminated by dialysis overnight at 4°C against 1,000 volumes of 50 mM Tris-HCl (pH 7.3), 1 mM EDTA, and 10 mM β-mercaptoethanol. Finally, the purified enzyme was precipitated with $(NH_4)_2SO_4$ at 60% saturation and stored in liquid N_2. Under this condition, the enzyme was stable for at least 6 months. Purified enzyme had a specific activity of 22 to 25 $\mu mol \cdot min^{-1} \cdot mg^{-1}$

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[3] Abbreviations: PEP, phosphoenolpyruvate; MOPS, 4-morpholinepropanesulphonic acid.

Table I. *Modulation of PEP Carboxylase Activity by Light and Thiol Compounds*

PEP carboxylase was extracted from maize leaves maintained in darkness or preilluminated at 25°C for 30 min by a 150-w tungsten lamp through 2 cm of a 1% solution of $CuSO_4$ as heat-absorbing filter, essentially as described in the text except that β-mercaptoethanol was omitted. The leaf (1 g) was rapidly cut into pieces and homogenized at 0°C for 45 s in a high speed homogenizer in 10 ml of 100 mM Tris-HCl (pH 7.3) and 4 mM EDTA. Cellular debris was eliminated by filtration through a nylon cloth and the filtrate centrifuged at 12,000*g* in an Eppendorf microcentrifuge for 30 s. The PEP carboxylase activity of the crude extract was determined in 50-μl aliquots of the supernatant. The PEP carboxylase purified and kept as described in the text was desalted by centrifugation through Sephadex G-50 column equilibrated with 50 mM MOPS-NaOH (pH 7) plus or minus 10 mM β-mercaptoethanol before the assay of the activity.

Experiment		PEP Carboxylase Activity
		μmol min⁻¹ mg⁻¹ protein
	Crude extract from	
1	Darkness leaves	0.12
2	1; then DTT (50 mM)	0.20
3	Preilluminated leaves	0.19
4	3; then DTT (50 mM)	0.19
	Purified enzyme desalted	
5	In the presence of β-mercaptoethanol	24.6
6	In the absence of β-mercaptoethanol	12.6
7	6; then DTT (50 mM)	23.3

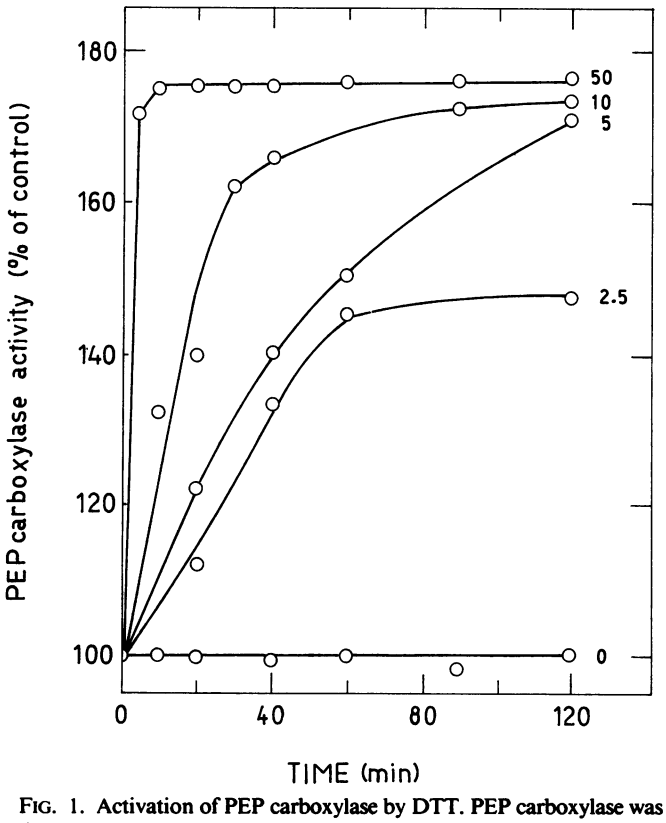

FIG. 1. Activation of PEP carboxylase by DTT. PEP carboxylase was preincubated at 25°C with the concentration of DTT stated (figures on the slope, mM) as described in the text. At the indicated times, aliquots were withdrawn and assayed for activity.

protein and migrated as a single protein band in polyacrylamide gel electrophoresis, although some minor bands (about 5%) were also observed.

Enzyme activity was determined spectrophotometrically at 30°C monitoring NADH oxidation at 340 nm in a UNICAM SP 1800B spectrophotometer by coupling the PEP carboxylase reaction with malic dehydrogenase. The standard assay medium contained 50 mM Tris-HCl (pH 8), 5 mM $MgCl_2$, 0.15 mM NADH, 10 mM $NaHCO_3$, 2 IU malic dehydrogenase, 4 mM PEP, and PEP carboxylase (4 μg of protein) in a total volume of 1 ml.

The enzyme activity was also assayed following oxalacetate formation at 272 nm using an ε of 910 $M^{-1} \cdot cm^{-1}$ (8, 27). The assay medium was the same as the above described except that NADH and malic dehydrogenase were omitted.

Protein Measurement. Total protein concentration was determined by colorimetric method of Lowry *et al.* (11) or alternatively by the Coomassie Brilliant Blue binding method (23), using BSA as standard.

DTT Treatment of PEP Carboxylase. Enzyme (1 μM) was incubated with different concentrations of DTT in 50 mM MOPS-NaOH (pH 7) at 25°C. Aliquots were withdrawn at different times and diluted 1000-fold in the assay medium. Controls were similarly treated except that DTT was absent. The addition of DTT (1000-fold diluted) in the assay medium had no effect on the enzyme activity. In some experiments, the excess of DTT was removed from the preincubation medium according to Penefsky (19).

Titration of Accessible and Free Sulfhydryl Groups of PEP Carboxylase. Radiochemical titration of sulfhydryl groups was performed by incubation of PEP carboxylase (0.5 mg of protein/ml) with 5 mM *N*-[^{3}H]ethylmaleimide (9.1×10^6 cpm/ml) for 2 h at 25°C in a medium (0.3 ml) containing 100 mM MOPS-NaOH (pH 7) and 1 mM EDTA. Then SDS (1%) or urea (8 M) was added and the mixture was further incubated for 2 h. Finally, the excess of *N*-[^{3}H]ethylmaleimide was eliminated by two consecutive centrifugations in Sephadex G-50 fine columns (19) equilibrated with 100 mM MOPS-NaOH (pH 7) and 1 mM EDTA. Protein concentration and *N*-[^{3}H]ethylmaleimide incorporation were determined in aliquots of the desalted protein.

Radioactivity incorporated was determined in a Beckman LS 8100 liquid scintillation counter using a mixture of 0.25% 2,5-diphenyloxazole, 10% naphthalene in dioxane as the scintillation cocktail.

A mol wt of 400,000 (29) was used to determine the molar concentration of PEP carboxylase.

RESULTS

The activity of PEP carboxylase in a crude extract that was rapidly obtained from illuminated maize leaves ('light' activity) was about 65% higher than the activity of the enzyme in crude

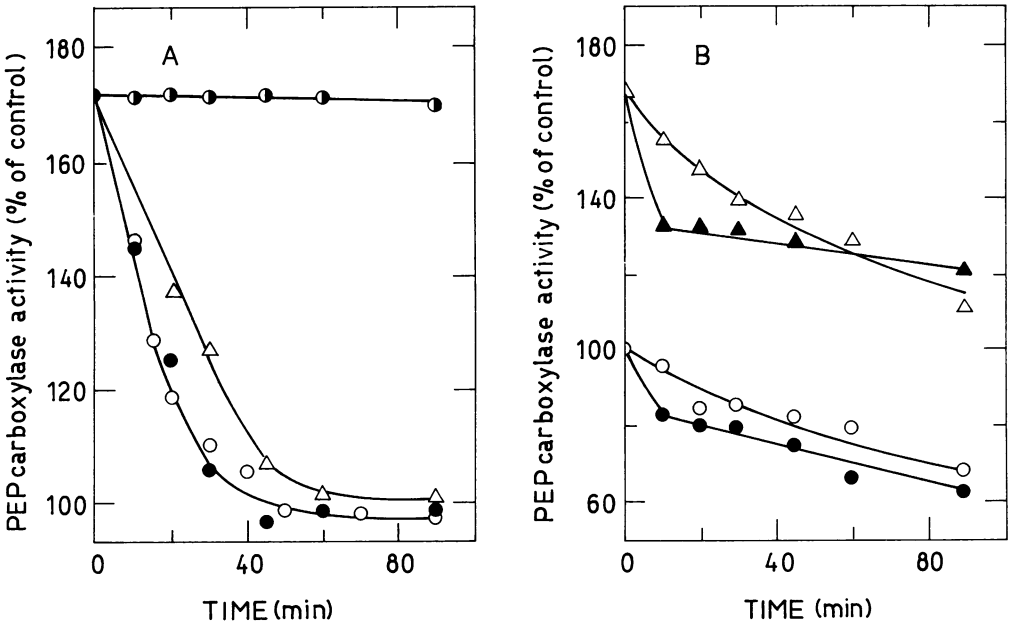

FIG. 2. Reversion of PEP carboxylase activation by diamide and iodoacetate. A, The enzyme was incubated for 90 min at 25°C with 10 mM DTT. The activation process was terminated by centrifugation through Sephadex G-50 column and PEP carboxylase activity determined as described in the text. After centrifugation, the activated enzyme was incubated at 25°C for the time stated without addition (△); with 5 mM EDTA (◑); 10 mM diamide in the absence (●) or in the presence (○) of 5 mM EDTA. B, Control and DTT-activated enzymes were incubated with 10 mM iodoacetamide (○, △) or 10 mM iodoacetate (●, ▲). At the time stated, the enzymic activity was assayed as described in the text.

Table II. *Titration of Accessible and Free Sulfhydryl Groups of PEP Carboxylase*

PEP carboxylase was incubated at 25°C for 60 min with the reagents shown. The reagent was eliminated by centrifugation through Sephadex columns before the following treatment and determination of activities. The number of sulfhydryl groups available in denatured (free groups) and nondenatured (accessible groups) PEP carboxylase was determined by *N*[^{3}H]ethylmaleimide incorporation as described in the text. Values shown are means from three experiments.

Exp.	Treatment of PEP Carboxylase	PEP Carboxylase Activity	Sulfhydryl Groups
		μmol min^{-1} mg^{-1} protein	*mol NEM/mol enzyme*[a]
	Accessible groups		
1	None	12.9	8
2	DTT (50 mM)	25.7	12
3	2; then diamide (10 mM)	12.1	10
	Free groups		
4	SDS (1%)		12
5	SDS (1%) plus DTT (50 mM)		16
6	Urea (8 M)		12
7	Urea (8 M) plus DTT (50 mM)		16

[a] Values are rounded to the nearest whole number.

extract from leaves kept in the dark ('dark' activity) (Table I). The dark activity was enhanced until the level of the activity from illuminated leaves when it was incubated with 50 mM DTT during 5 min at 25°C, while the light activity was not affected by a similar incubation with DTT.

A similar difference was observed when the activity of the purified PEP carboxylase was assayed after filtration through Sephadex G-50 column equilibrated with 50 mM MOPS-NaOH (pH 7) with or without 10 mM β-mercaptoethanol. In this case, the activity of the purified enzyme desalted in the absence of β-mercaptoethanol was stimulated by the incubation with 50 mM DTT (Table I).

Different rates of PEP carboxylase activity were obtained when the purified enzyme was incubated with various concentrations of DTT at 25°C (pH 7) in the absence of EDTA (Fig. 1). The activity of control enzyme was stable during the preincubation time (Fig. 1). Similarly, other thiol compounds such as β-mercaptoethanol, cysteine, DTT, and 2,3-dimercaptopropanol activated purified PEP carboxylase in a time-dependent manner (not shown).

The activity of PEP carboxylase obtained after incubation of the enzyme with DTT was 66 ± 6% (in 12 experiments) higher than control activity. The presence of $MgCl_2$ did not alter the stimulation induced by thiol compounds (not shown).

The rate of PEP carboxylase activation appears to be dependent on the reducing conditions of the reaction medium. After removal of the reducing agent, the activity diminished to the initial level (Fig. 2A) and was reduced faster by the addition of diamide, a specific oxidant of vicinal dithiols (9), in the presence or absence of EDTA. On the other hand, EDTA stabilized the maximal PEP carboxylase activity (Fig. 2A) presumably by chelating traces of heavy metal present in the medium. Low concentrations of $CuCl_2$ or $CdCl_2$ totally inhibited PEP carboxylase activity (6).

These results clearly suggest that the activation of PEP carboxylase by DTT may be mediated by reduction of disulfide bonds. Diamide may be able to eliminate the activation by oxidation of the newly formed vicinal dithiols. Blocking these groups with

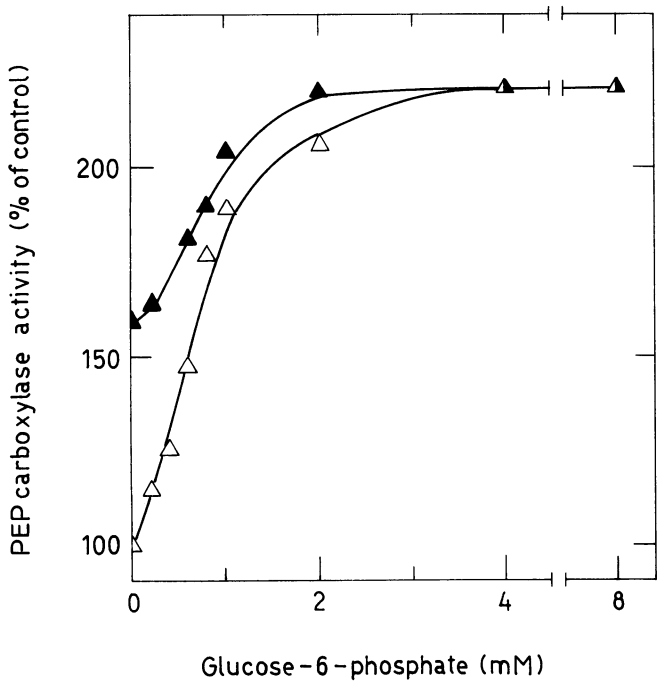

FIG. 3. Activation of control and DTT-treated enzyme by glucose-6-P. The enzymic activities of control (△) and DTT-activated (▲) enzymes were assayed as described in the text with or without addition of the stated concentration of glucose-6-P.

iodoacetamide or iodoacetate would prevent oxidation and reversion of activation. Figure 2B shows that this is indeed the case. The incubation of DTT-activated PEP carboxylase with iodoacetamide or iodoacetate resulted in a lower rate of loss of enzyme activity (Fig. 2B). Moreover, this loss of activity presumably is a consequence of the modification of some of the thiol groups of the enzyme (1, 14, 25, 26) which would be exposed to the reagent in both forms of it. Thus, the activity of PEP carboxylase may be modulated by the reversible reduction-oxidation of disulfide bonds.

Table II shows the titration of accessible and free thiol groups of the PEP carboxylase. Titration of accessible thiols of the enzyme by labeling with *N*-[^{3}H]ethylmaleimide shows an increase from 8 to 12 thiols/mol of PEP carboxylase after activation by DTT (Table II, experiments 1 and 2). The subsequent treatment of the DTT-activated enzyme with diamide partially reversed the change induced by DTT (Table II, experiment 3), resulting in the disappearance of two of four new thiol groups exposed during the preincubation with DTT. Thus, the activation of the PEP carboxylase by DTT can be visualized as a consequence of the reduction of two disulfide bonds. Apparently, the oxidation to a disulfide bond of two of the four new thiols is sufficient to reverse activation.

The number of free sulfhydryl groups of PEP carboxylase was determined by incorporation of *N*-[^{3}H]ethylmaleimide to the enzyme denatured by 1% SDS or 8 M urea (Table II, experiments 4 and 6). Under these conditions, we found 12 free thiols/mol of the enzyme. However, when the PEP carboxylase was denatured in the presence of DTT, the number of thiol groups blocked by *N*-[^{3}H]ethylmaleimide was 16 (Table II, experiments 5 and 7). These results suggest that the PEP carboxylase has a total of 16 thiol groups/mol, four of them involved in the formation of two disulfide bonds (*cf.* lines 4 and 6 with lines 5 and 7, Table II). From the 12 free thiols, eight are accessible and the other four are not (*cf.* line 1 with lines 4 and 6, Table II).

The activation of PEP carboxylase by DTT does not affect the affinity of the enzyme for its substrates. The values of K_m for PEP (0.4 mM), $MgCl_2$ (0.9 mM), and bicarbonate (0.6 mM) did not change upon activation of the enzyme by DTT while the V_{max} was increased by a factor of 1.65.

Organic acids like malate and oxalacetate were reported to be inhibitors of the PEP carboxylase (5, 10, 20, 21, 28). The effects of these negative modulators on the DTT-activated enzyme did not differ from those obtained for the native enzyme since they yielded similar I_{50} values (malate, 5 mM; oxalacetate, 0.35 mM).

Figure 3 shows that the DTT-activated enzyme could be activated by glucose-6-P, a positive modulator of PEP carboxylase (2). Although the degree of the activation induced by glucose-6-P is markedly different in control and activated enzymes, the concentration of glucose-6-P necessary to achieve 50% of maximal activation was 0.8 mM regardless of the state of the enzyme. This result suggests that the DTT and glucose-6-P effects are not additive.

DISCUSSION

PEP carboxylase activity from maize was stimulated upon exposure of the purified enzyme to DTT at room temperature (Fig. 1). The activation did not require divalent cation such as Mg^{2+} and was totally reversed by diamide (Fig. 2).

Titration of thiol groups of the PEP carboxylase shows that it has 16 groups per mol of enzyme, four of them involved in the formation of two disulfide bonds and the other 12 as free thiols (Table II). From the 12 free thiols, eight were accessible and the other four hidden to sulfhydryl reagents.

The number of accessible thiols per mol of PEP carboxylase increased from 8 to 12 after treatment with DTT (Table II), suggesting that the reduction of two disulfide bonds is associated with the stimulation of the enzyme. Moreover, the reoxidation of a DTT-exposed vicinal dithiol by diamide resulted in a loss of the activation (Table II).

The DTT-activated enzyme showed the same affinity for its substrates but the maximal activity increased by a factor of 1.65. On the other hand, the activated form of the enzyme had the same sensitivity with respect to the modulators, malate, oxalacetate, and glucose-6-P, although a significant difference in the magnitude of the activation afforded by the latter on both forms of the enzyme was observed (Fig. 3). The relatively smaller activation produced by glucose-6-P on DTT-activated enzyme can be interpreted assuming that the allosteric site for this ligand is partially altered.

Changes in the redox state of disulfide bridges in PEP carboxylase may be relevant to the regulation of this enzymic activity *in vivo*. Of particular interest is the observation made by Slack (24) on the physiological activation by light of PEP carboxylase from *Amaranthus palmeri*. We found a similar activation by light in the activity of PEP carboxylase in crude extracts rapidly obtained from illuminated maize leaves (Table I). Moreover, the activity of PEP carboxylase in crude extracts obtained from leaves previously adapted to the dark was enhanced until the level of the activity of the illuminated leaves by a short incubation with DTT while the latter was not affected by a similar treatment (Table I). The near 2-fold stimulation by light reported by Slack (24) and by us (this paper) is coincident with the stimulation by DTT described for the purified enzyme when it was desalted in the absence of β-mercaptoethanol (Table I; Fig. 1). Thus, it is conceivable that the light stimulation of PEP carboxylase *in vivo* can occur through a mechanism involving reversible reduction of disulfide bridges.

LITERATURE CITED

1. BANDURSKI RS 1955 Further studies on the enzymatic synthesis of oxalacetate from phosphoenolpyruvate and carbon dioxide. J Biol Chem 217: 137–150
2. COOMBS J, CW BALDRY, C BUCKE 1973 The C_4 pathway in *Pennisetum purpureum*. The allosteric nature of PEP carboxylase. Planta 110: 95–107

3. COOPER TG, HG WOOD 1971 The carboxylation of phosphoenolpyruvate and pyruvate. The active species of "CO_2" utilized by phosphoenolpyruvate carboxylase and pyruvate carboxylase. J Biol Chem 246: 5488–5490
4. HATCH MD, JR OLIVER 1978 Activation and inactivation of phosphoenolpyruvate carboxylase in leaf extracts from C_4 species. Aust J Plant Physiol 5: 571–580
5. HUBER SC, GE EDWARDS 1975 Inhibition of phosphoenolpyruvate carboxylase from C_4 plants by malate and aspartate. Can J Bot 53: 1925–1933
6. IGLESIAS AA, CS ANDREO 1984 Reversible inhibition of *Zea mays* phosphoenolpyruvate carboxylase by copper and cadmiun ions. Photosynthetica. In press
7. IGLESIAS AA, CS ANDREO 1983 The presence of essential histidyl residues in phosphoenolpyruvate carboxylase from maize leaves. Biochim Biophys Acta 749: 9–17
8. JONES R, MB WILKINS, CA FEWSON, ADB MALCOM 1978 Phosphoenolpyruvate carboxylase from the crassulacean plant *Bryophyllum fedtschenkoi* Hamet et Perrier. Biochem J 175: 391–406
9. KOSOWER NS, EM KOSOWER, B WERTHEIM, W CORREA 1969 Diamide a new reagent for the intracellular oxidation of glutathione to the disulfide. Biochem Biophys Res Commun 37: 593–596
10. LOWE S, CR SLACK 1971 Inhibition of maize leaf phosphoenolpyruvate carboxylase by oxalacetate. Biochim Biophys Acta 235: 207–209
11. LOWRY O, N ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
12. MANETAS Y, NA GAVALAS 1982 Evidence for essential sulfhydryl group(s) in photosynthetic phosphoenolpyruvate carboxylase: protection by substrate, metal-substrate and glucose-6-phosphate against *p*-chloromercuribenzoate inhibition. Photosynthetica 16: 59–66
13. MANETAS Y, NA GAVALAS 1983 Reduced glutathione as an effector of phosphoenolpyruvate carboxylase of the Crassulacean acid metabolism plant *Sedum praealtum* D.C. Plant Physiol 71: 187–189
14. MARUYAMA H, MD LANE 1962 Purification and properties of phosphoenolpyruvate carboxylase from the germinating peanut cotyledon. Biochim Biophys Acta 65: 207–218
15. MUKERJI SK 1977 Corn leaf phosphoenolpyruvate carboxylases. Purification and properties of two isoenzymes. Arch Biochem Biophys 182: 343–351
16. O'LEARY MH 1982 Phosphoenolpyruvate carboxylase: an enzymologist's view. Annu Rev Plant Physiol 33: 297–315
17. O'LEARY MH, JE RIFE, JD SLATER 1981 Kinetic and isotope effect studies of maize phosphoenolpyruvate carboxylase. Biochemistry 20: 7308–7314
18. OSMOND CB 1978 Crassulacean acid metabolism: A curiosity in context. Annu Rev Plant Physiol 29: 379–414
19. PENEFSKY H 1977 Reversible binding of Pi by beef heart mitochondrial adenosine triphosphatase. J Biol Chem 252: 2891–2899
20. RAGHAVENDRA AS, VSR DAS 1975 Malonate inhibition of allosteric phosphoenolpyruvate carboxylase from *Setaria italica*. Biochem Biophys Res Commun 66: 160–165
21. RAGHAVENDRA AS, VSR DAS 1976 Phosphoenolpyruvate carboxylase from *Setaria italica*: Inhibition by oxalacetate and malate. Z Pflanzenphysiol 78: 434–437
22. RAGHAVENDRA AS, RH VALLEJOS 1982 Regulation of phosphoenolpyruvate carboxylase from C_4 plants: Involvement of thiol groups in catalytic reaction of enzyme from *Amaranthus viridis*. Indian J Exp Biol 20: 619–622
23. SEDMAK J, S GROSSBERG 1977 A rapid, sensitive and versatile assay for protein using Coomassie Brilliant Blue G 250. Anal Biochem 79: 544–552
24. SLACK CR 1968 The photoactivation of a phosphopyruvate synthase in leaves of *Amaranthus palmeri*. Biochem Biophys Res Commun 30: 483–488
25. SMITH TE 1968 Partial purification and characteristics of potato phosphoenolpyruvate carboxylase. Arch Biochem Biophys 125: 178–188
26. TCHEN TT, B VENNESLAND 1955 Enzymatic carbon dioxide fixation into oxalacetate in wheat germ. J Biol Chem 213: 533–546
27. TING IP 1968 CO_2 metabolism in corn roots. Inhibition of P-enolpyruvate carboxylase by L-malate. Plant Physiol 43: 1919–1924
28. TING IP, CB OSMOND 1973 Photosynthetic phosphoenolpyruvate carboxylases. Characteristics of alloenzymes from leaves of C_3 and C_4 plants. Plant Physiol 51: 439–447
29. UEDAN K, T SUGIYAMA 1976 Purification and characterization of phosphoenolpyruvate carboxylase from maize leaves. Plant Physiol 59: 906–910