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# Photoregulation of Phosphoenolpyruvate Carboxylase in *Salsola soda* L. and Other $C_4$ Plants[1]

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## ABSTRACT

**Photoactivation of phosphoenolpyruvate carboxylase was found to occur in several, though not all, $C_4$ species examined; *Salsola soda* L. was used for a detailed study of this effect of light.**

**Activity differences between light and darkness are maximized when glycerol (25% v/v) is included in the extraction medium and in the absence of mercaptoethanol. In plants grown in the growth chamber, the night-form of the enzyme, in addition to low activity, shows a positive cooperativity (with phosphoenolpyruvate), which is gradually abolished by light of increasing intensities. This allosteric behavior is absent in plants adapted to a high light environment. Activation and deactivation, under light and darkness respectively, are quite fast, suggesting post-translational regulation. The photoactivation appears to depend on photosynthetic electron flow, since it is saturated at high photon fluxes (around 1000 microeinsteins per square meter per second) and inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea.**

A large number of enzymes involved in photosynthetic carbon reduction and flow are subject to post-translational regulation by light (1, 5) in a manner ensuring maximum efficiency in carbon fixation, reduction and use. Though most of these enzymes are chloroplastic, some cytoplasmic ones are also affected by light (3, 13).

Phosphoenolpyruvate carboxylase (PEPCase[3]), a key cytoplasmic (10, 23) enzyme of the $C_4$ pathway, is not included in the above group, though a 2-fold activation by light has been incidentally reported in a paper concerning pyruvate, $P_i$ dikinase in *Amaranthus palmeri* leaves (26). Previous work in our laboratory implicated sulfhydryl group(s) in PEPCase activity (9, 18) and suggested that a reductive photoactivation of this enzyme was probable (8, 17, 18); preliminary evidence concerning such an effect of light in *Atriplex tatarica* L. was also obtained (8). Pursuing this lead, we screened several $C_4$ species for PEPCase photoactivation and studied in more detail this phenomenon in *Salsola soda* L., a halophyte of Chenopodiaceae, which appeared most promising for our purpose.

*Salsola soda* L. is a $C_4$ species (25), though the anatomical features of its succulent leaves are quite unconventional. A central water storage tissue is lined by two distinct monolayers of chlorenchyma; the inner layer consists of closely packed cells and contains minute bundles, whereas the thin-walled cells of the outer layer are loosely arranged and apparently play the role of mesophyll cells, as shown in *Suaeda monoica* (24). This anatomy is quite similar to that of *Salsola kali* L. (22) and common in a number of succulent Chenopodiaceae (25).

## MATERIALS AND METHODS

Plants were grown from seeds in a growth chamber with temperature, RH and light/dark cycles of 28/18°C, 40/70% and 10.5/13.5 h, respectively. Irradiance at plant level was around 250 $\mu E/m^2 \cdot s$ PAR, given by a mixture of fluorescent and incandescent lamps. In some cases, cited in the text, plant material was collected from the field.

For enzyme extraction, 500 mg of mature leaves were ground in a prechilled mortar with purified sea sand and 5 ml of standard extraction medium (0.1 M Tris-HCl [pH 7.7], 1 mM EDTA, 10 mM $MgCl_2$, 25% v/v glycerol, 3% w/v PVP plus a small amount of insoluble polyvinylpolypyrrolidone. Cases where mercaptoethanol (Et-SH) was included or glycerol omitted are specified in the text. The extract was centrifuged for 1 min and the clear supernatant was used either immediately (crude extract) or desalted through a 12- × 1-cm Sephadex G25 column equilibrated with 0.1 M Tris-HCl [pH 7.7] in 25% v/v glycerol (desalted extract). All above steps were carried out at 4°C.

Assays of PEPCase activity were run at 30°C in 3 ml final volume of 0.1 M Tris-HCl [pH 8.2], 1 mM $NaHCO_3$, 5 mM $MgCl_2$, 0.14 mM NADH, 4.5 units of malate dehydrogenase (pig heart, Sigma) and PEP as specified. The reaction was started with the addition of enzyme and its rate was measured by the decrease in absorbance at 340 nm (oxidation of NADH). Since the activity declined rapidly during the assay, particularly at low PEP concentrations, the true initial activities were calculated by mathematical extrapolation to zero time (9).

In assessing the effect of DCMU, detached leaves of *Salsola soda* were floated on a 10 $\mu$M DCMU solution (aqueous + 1‰ v/v ethanol) for 2 h in the dark (25°C) and 1.5 h in the light (30°C) at a photon flux of 1700 $\mu E/m^2 \cdot s$; controls without DCMU were run concurrently. Extractable activity of PEPCase was assayed at the end of the dark period and after the light treatment. The effect of DCMU on photosynthetic activity was determined by measuring $CO_2$ exchanges in light and darkness with an IR gas analyzer working in a closed system.

## RESULTS AND DISCUSSION

**Effects of Glycerol and Et-SH on PEPCase Activity and Stability.** In our attempt to compare PEPcase activities extracted at daytime or during the night, we decided to avoid the inclusion of a thiol in the extraction medium, since preliminary results had shown that a difference in activities may be partly or entirely masked by thiols (8). However, when mercaptoethanol is omitted from the extraction medium, PEPCase of *Salsola soda* is quickly inactivated and detailed studies of the enzymic behavior cannot

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[2] Doctoral thesis.

[3] Abbreviations: PEPCase, phosphoenolpyruvate carboxylase; PEP, phosphoenolpyruvate; Et-SH, mercaptoethanol.

be pursued. We tried, therefore, to stabilize the activity with glycerol, which was shown in the past to exert such an effect (4, 16, 21, 27). As anticipated, glycerol, even in the absence of a thiol, stabilized PEPCase activity to a considerable extent (Fig. 1); in addition, the data strongly suggested that the activities obtained with glycerol are more representative of the situation *in vivo*, since the curves with or without glycerol could be extrapolated to comparable values at zero time.

A comparison of the results obtained with different extraction media (Table I) clearly shows that activity differences between night and day are maximized when glycerol is used, whereas Et-SH narrows the difference, mainly in the presence of glycerol. Both night and day activities are increased with Et-SH, but this effect is much stronger on night activities, so that the overall difference becomes smaller. This effect of Et-SH is almost complete at 10 mM; only small increases in activity (11–17%) are obtained by raising the Et-SH concentration up to 50 mM.

The data are consistent with the hypothesis that *in vivo* PEPCase is reversibly activated-deactivated through reduction (in light)-oxidation (in darkness) of sulfhydryl groups (8, 17, 18); when a thiol is added, the enzyme is activated during extraction and the *in vivo* differences in activity are masked. An alternative interpretation is also conceivable: the observed night-day difference in activities might be an artifact, due to faster inactivation of the enzyme, when extracted during the night; Et-SH slows down this inactivation decreasing thereby the magnitude of the artifact. This explanation, however, is negated, if not rigidly excluded, by the results obtained with glycerol, (Fig. 1), which both stabilizes the enzyme and maximizes the night-day activity differences.

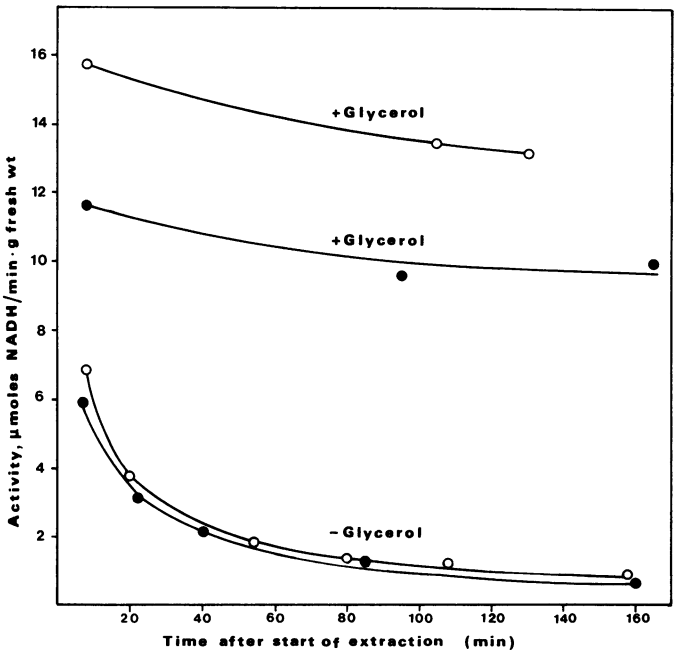


FIG. 1. Stabilization of PEPCase activity (PEP at 1.82 mM) by glycerol (25% v/v) in the absence of Et-SH; activity differences between night (●) and day (○) can be recognized only in the presence of glycerol. Plant: *Salsola soda* L.; irradiance at 300 $\mu E/m^2 \cdot s$ PAR.

Based on the above results we proceeded to a more detailed study of the light effect on extractable PEPCase activity and behavior, using glycerol (25% v/v) and omitting mercaptoethanol from the extraction medium.

**Effects of Light on PEPCase Activity and Behavior.** The results of a representative experiment on the effects of light are shown as double reciprocal plots in Figure 2. *Salsola soda* leaves were extracted either at the end of the dark period or 2 h after the plants were transferred to the respective light level. Under darkness, the enzyme exhibits a clearly allosteric behavior, which is weakened under low light and transformed to almost Michaelis-Menten at the higher photon flux. This general behavior was invariably obtained in all replications with plants grown in the growth chamber, though the levels of extractable activity and the apparent degree of allostericity varied considerably. The same phenomenon was observed with either crude or desalted extracts.

Since plants grown under low or high light intensities exhibit substantial differences in enzymic activities (12), we also used *Salsola* plants grown in the open (PAR up to 2000 $\mu E/m^2 \cdot s$). PEPCase activity was again increased in the light, but the allostericity was absent from the less active 'dark' enzyme. The same loss of allostericity was also observed when Et-SH was included in the extraction medium. Obviously, the effects of environmental conditions of growth on PEPCase properties warrant a more detailed and extensive study in the future.

Progress towards an understanding of the functional behavior of PEPCase under light or darkness is apparently hindered by changes in enzymic properties occurring during extraction. The tacit assumption that extractable activity gives a good measure of the situation *in vivo* is not always valid and this seems to be the case with $C_4$-PEPCase, as it is with CAM-PEPCase (16, 29). An extraction method stabilizing the enzyme and conserving, as closely as possible, its *in vivo* conformation is badly needed, if a true picture of the photoregulatory mechanism is to be gained.

**Kinetics of PEPCase Activation and Inactivation.** The increase in extractable PEPCase activity under light is quite fast and takes place without a detectable lag (Fig. 3). Equally fast appears to be the inactivation of the enzyme after a light/dark transition, if the activity is assayed at low PEP levels. At high (1.82 mM) PEP concentrations (not shown), the fall in activity is much slower than the increase, suggesting that the induction of positive cooperativity may be the primary effect of darkness.

This oscillation in PEPCase activity cannot be ascribed to a circadian rhythm since the light and darkness were imposed at times remote from the programmed light/dark and dark/light

Table I. *Effects of Light on PEPCase Activities Extracted with or without Glycerol or ET-SH*

Enzymic source: 100 μl crude extract from *Salsola soda* leaves; activity assayed 8 min after start of extraction; plants under 2000 $\mu E/m^2 \cdot s$ PAR for more than 1.5 h or in the dark for about 13 h.

| [PEP] | PEPCase Activity | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Extraction buffer A[a] | | | Extraction buffer B | | | Extraction buffer C | | | Extraction buffer D | | |
| | Dark | Light | % Increase | Dark | Light | % Increase | Dark | Light | % Increase | Dark | Light | % Increase |
| *mM* | *μmol NADH/min·g fresh wt* | | | | | | | | | | | |
| 0.49 | 8.4 | 17.5 | 108 | 14.2 | 27.4 | 93 | 9.6 | 27.3 | 184 | 18.8 | 30.2 | 61 |
| 0.97 | 12.4 | 23.1 | 86 | 19.2 | 35.0 | 82 | 14.8 | 29.5 | 99 | 23.9 | 38.6 | 61 |
| 1.82 | 15.6 | 24.7 | 58 | 24.7 | 38.8 | 57 | 19.7 | 36.8 | 87 | 29.2 | 38.8 | 33 |

[a] A, Without glycerol or Et-SH; B, A + 10 mM Et-SH; C, A + 25% v/v glycerol; D, A + Et-SH + glycerol.

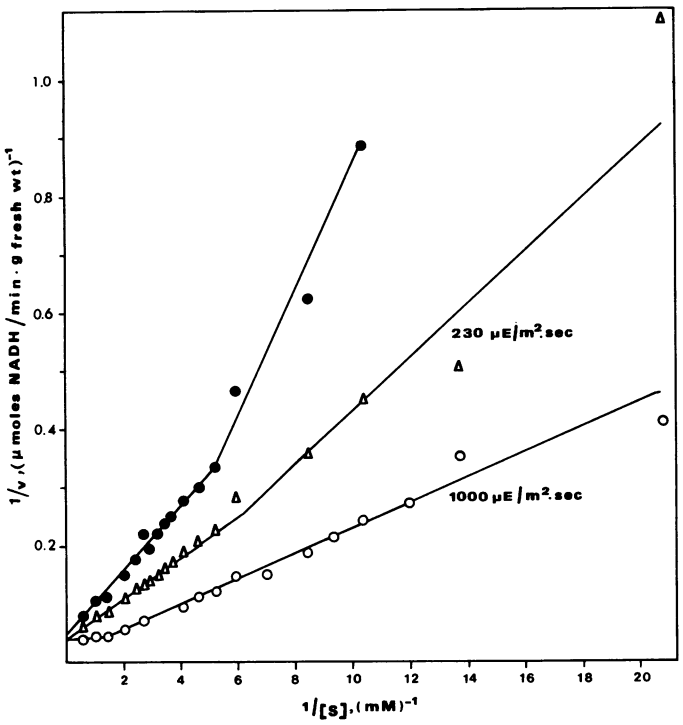


FIG. 2. Lineweaver-Burk plots of reciprocal PEPCase activity vs $[PEP]^{-1}$; *Salsola soda* L. plants grown in the growth chamber were used as the enzymic source. Leaves for extraction were taken during darkness (●) or at two levels of PAR.

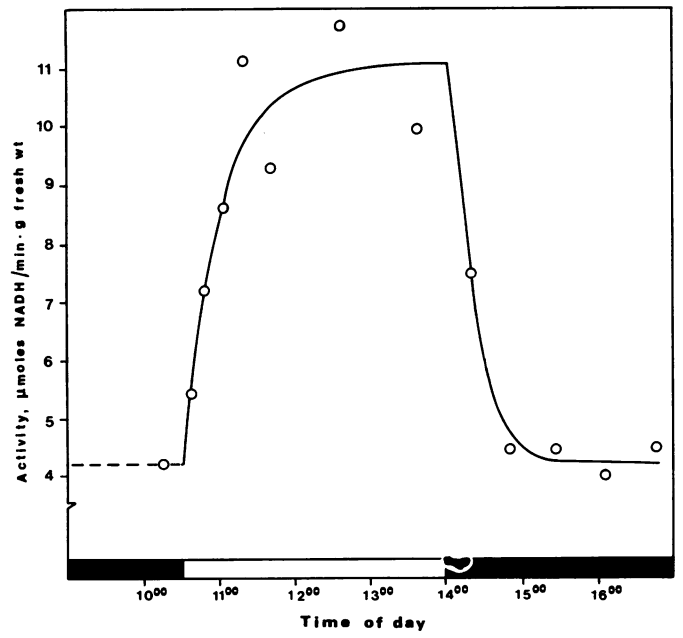


FIG. 3. Activation/deactivation of PEPCase activity in *Salsola soda* L. leaves during an imposed dark/light/dark cycle; darkness and light are shown in the abscissa as black or white bars, respectively. Irradiance at 2000 $\mu E/m^2 \cdot s$ PAR; PEP at 0.24 mM.

transitions, under which the plants were grown in the growth chamber.

The fast activity changes and the lack of lag periods constitute strong evidence that the effects of light and darkness are post-translational; a change in the conformational or polymeric state of the enzymic molecule, under the influence of an effector generated and maintained by light, appears as the most plausible hypothesis for further study. The activating effect of Et-SH and the implication of sulfhydryl groups in PEPCase activity (9, 18) suggest that the responsible effector could be a reductant generated by photosynthetic electron flow, as it is the case with other photosynthetic enzymes (2, 6, 30).

**PEPCase Activation *versus* Irradiance Level.** The effect of increasing light intensity on PEPCase activation was studied in a series of experiments at photon fluxes up to 2000 $\mu E/m^2 \cdot s$. Enzymic activity was assayed at three substrate (PEP) concentrations just before the onset of light and 1.5 h after the start of each irradiance; such an illumination period was necessary for full activation, as shown previously (Fig. 3).

Since extractable activity usually varies considerably among individual plants, only one potted plant was used in each experiment and the light treatments were given consecutively, starting with the lower intensity. Irradiances up to 230 $\mu E/m^2 \cdot s$ were given in the growth chamber and higher ones in the sunlight, using layers of cheesecloth to obtain the desired light intensity.

Photon fluxes up to 800 $\mu E/m^2 \cdot s$ were used initially without reaching light saturation in PEPCase activation; thus, in subsequent experiments, the irradiances were augmented up to 2000 $\mu E/m^2 \cdot s$. The results of a representative experiment are shown in Figure 4; PEPCase activation appears to be light saturated at a photon flux around 1000 $\mu E/m^2 \cdot s$, *i.e.* at about half the intensity of full sunlight. It should be noted, however, that the plants used in these experiments were grown in the growth chamber at a maximum irradiance of only 230 $\mu E/m^2 \cdot s$. It is conceivable, therefore, that PEPCase activation might be light saturated at even higher photon fluxes in *Salsola* plants grown in the open and adapted to higher photon fluxes.

An activation of PEPCase by blue light in colorless mutant *Chlorella* (14) and also in wild-type *Chlorella* (20) has been reported, but this effect was saturated at extremely low light intensities, *i.e.* at 300 $erg/cm^2 \cdot s$, corresponding to about 1.15 $\mu E/m^2 \cdot s$ at the wavelength used (456 nm). Even if we take into account that only a small percentage of the white light used by us might be active and that it could be further attenuated by Chl absorption, the light saturation level observed in *Salsola* would remain more than one order of magnitude higher than that reported in *Chlorella* cells. Therefore, it seems reasonable to infer

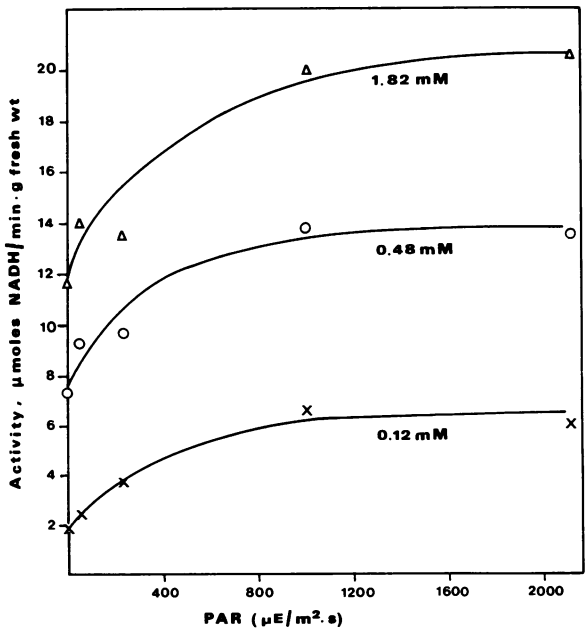


FIG. 4. PEPCase activity in *Salsola soda* L. leaves, assayed at three levels of PEP, as a function of light intensity.

that we are dealing with a different phenomenon. The high light saturation level in *Salsola* points again towards a mechanism connected with the photosynthetic process.

**Inhibition by DCMU of PEPCase Light Activation.** To obtain evidence concerning the involvement of photosynthetic electron flow, we examined the effect of DCMU on light activation of PEPCase. With preliminary experiments, we had ascertained that photoactivation occurred even in detached leaves, though the activities obtained under light were considerably lower than those found in leaves taken directly from illuminated plants. The floating method (see "Materials and Methods") was selected because spraying of intact plants with DCMU solutions did not inhibit the photosynthetic $CO_2$ absorption in the light; apparently the DCMU applied on leaf surfaces was not taken up by the plants. On the other hand, DCMU-treated leaves with the floating method showed $CO_2$ evolution in the light roughly equal to dark respiration, whereas the controls were actively absorbing $CO_2$.

The results of three experiments are shown in Table II as means ± SD. It is evident that DCMU abolishes the light activation when the activity of PEPCase is assayed at a high substrate (PEP) level and partly inhibits it when the assay is run at lower substrate concentrations. On the basis of these results, it is tempting to hypothesize that the increase in maximum activity is due to the light absorbed by the photosynthetic pigments, whereas the abolishment of the allosteric behavior is mediated by a nonphotosynthetic photoreceptor. A final conclusion, however, cannot be reached until unequivocal evidence, such as an action spectrum, becomes available.

**Light Effect on PEPCase of Other $C_4$ Species.** A survey of $C_4$ species for light activation of PEPCase showed that this effect is rather widespread, though not invariably present. Activation ranging from 20 to 110% over the activity values in the dark were found in *Atriplex tatarica* L., *Atriplex halimus* L., *Cyperus rotundus* L., *Salsola kali* L., *Digitaria sanguinalis* (L.) Scop, *Setaria verticillata* (L.) Beauv., *Eleusine indica* (L.) Gaetner, and *Portulaca oleracea* L.

On the other hand, no activation could be ensured under light in *Cynodon dactylon* (L.) Pers., *Saccharum ravenae* (L.) Murray, *Amaranthus* sp, *Zea mays* L., and *Sorghum bicolor* (L.) Moench. As a general rule, when the dark activity on a fresh weight basis is high, no further activation by light is obtained.

This apparent difference among $C_4$ species warrants a careful and detailed study before reaching a firm conclusion, since phenomena of activation/inactivation during extraction may be responsible for the observed diversity.

## CONCLUDING REMARKS

The data presented above show that PEPCase of many, if not all, $C_4$ plants should be added to the list of photosynthetic enzymes which are post-translationally regulated by light; an early indication of PEPCase photoregulation (26) had been largely ignored and not followed up, probably because the concurrently observed strong photoactivation of pyruvate, $P_i$ dikinase (11, 26) was considered adequate for an efficient regulation of the carbon flow in mesophyll cells. Indeed, such a concerted action of light on two successive enzymes in the same pathway seems redundant at first sight; in the case under consideration, however, it could be necessary, since PEP, as a branch point in intermediate metabolism (7), may arise from and be metabolized via different routes.

It is interesting to note that PEPCase of CAM is affected by light in the opposite way, *i.e.* it is turned off soon after the onset of illumination (16, 28, 29). Such a diametrically different effect of light may be due either to discrete regulatory sites in the two alloenzymes or to distinct effectors, generated by light in each case. The amply documented details (15, 16, 19, 28, 29) of the photoregulatory mechanism in CAM-PEPCase point towards the latter alternative, though the mechanism of photoactivation in $C_4$-PEPCase remains unknown. Preliminary evidence obtained in the course of our work (data not shown) suggests that the $C_4$-PEPCase is light activated only when stomata retain the ability to open and we suspect that the responsible effector may be an intermediate of the carbon flow; the elucidation of this point is the subject of our current effort.

Table II. *Inhibition of PEPCase Photoactivation by DCMU, Assayed at Three Substrate (PEP) Levels*

Light intensity at 1700 $\mu E/m^2 \cdot s$ PAR.

| [PEP] | PEPCase Activity[a] | | |
|---|---|---|---|
| | Dark | Light | Light + DCMU |
| *mM* | *μmol NADH/min·g fresh wt* | | |
| 0.49 | 4.4 ± 0.7 | 8.2 ± 0.6 | 6.1 ± 0.8 |
| 0.97 | 6.4 ± 0.7 | 10 ± 1.1 | 7.4 ± 1.4 |
| 1.82 | 9.8 ± 0.05 | 11.8 ± 1.0 | 9.1 ± 1.3 |

[a] Means of three replications ± SD.

## LITERATURE CITED


1. ANDERSON LE 1979 Interaction between photochemistry and activity of enzymes. *In* M Gibbs, E Latzko, eds, Encyclopedia of Plant Physiology, New Series, Vol 6, Photosynthesis II. Springer-Verlag, Berlin, pp 271–281
2. ANDERSON LE, M AVRON 1976 Light modulation of enzyme activity in chloroplasts. Generation of membrane-bound vicinal dithiol groups by photosynthetic electron transport. Plant Physiol 57: 209–213
3. ANDERSON LE, SC NEHRLICH 1977 Photosynthetic electron-transport system controls cytoplasmic glucose-6-phosphate dehydrogenase activity in pea leaves. FEBS Lett 76: 64–66
4. BHAGWAT AS, PV SANE 1976 Studies on enzymes of $C_4$ pathway: partial purification and kinetic properties of maize phosphoenolpyruvate carboxylase. Indian J Exp Biol 14: 155–158
5. BUCHANAN B 1980 Role of light in the regulation of chloroplast enzymes. Annu Rev Plant Physiol 31: 341–374
6. CHAMPIGNY M-L, E BISMUTH 1976 Role of photosynthetic electron transfer in light activation of Calvin cycle enzymes. Physiol Plant 36: 95–100
7. DAVIES DD 1979 The central role of phosphoenolpyruvate in plant metabolism. Annu Rev Plant Physiol 30: 131–158
8. GAVALAS NA, S CARAVATAS, Y MANETAS 1981 Sigmoid rate curves of photosynthetic ($C_4$) PEP carboxylase as an artifact of the assay: Factors affecting inactivation-reactivation of the enzyme. *In* G Akoyunoglou, ed, Photosynthesis, Vol 4. Balaban International Science Services, Philadelphia, pp 91–98
9. GAVALAS NA, S CARAVATAS, Y MANETAS 1982 Factors affecting a fast and reversible inactivation of photosynthetic phosphoenolpyruvate carboxylase. Photosynthetica 16: 49–58
10. HATCH MD, T KAGAWA 1973 Enzymes and functional capacities of mesophyll chloroplasts from plants with $C_4$-pathway of photosynthesis. Arch Biochem Biophys 159: 842–853
11. HATCH MD, CR SLACK 1969 Studies on the mechanism of activation and inactivation of pyruvate, phosphate dikinase. Biochem J 112: 549–558
12. HATCH MD, CR SLACK, TA BULL 1969 Light-induced changes in the content of some enzymes of the $C_4$-dicarboxylic acid pathway of photosynthesis and its effect on other characteristics of photosynthesis. Phytochemistry 8: 697–706
13. KACHRU RB, LE ANDERSON 1975 Inactivation of pea leaf phosphofructokinase by light and dithiothreitol. Plant Physiol 55: 199–202
14. KAMIYA A, S MIYACHI 1974 Effects of blue light on respiration and carbon dioxide fixation in colorless Chlorella mutant cells. Plant Cell Physiol 15: 927–937
15. KLUGE M, J BRULFERT, O QUEIROZ 1981 Diurnal changes in the regulatory properties of PEP-carboxylase in Crassulacean Acid Metabolism. Plant Cell Environ 4: 257–260
16. MANETAS Y 1982 Changes in properties of phosphoenolpyruvate carboxylase from the CAM plant *Sedum praealtum* DC. upon dark/light transition and their stabilization by glycerol. Photosynth Res 3: 321–333
17. MANETAS Y, NA GAVALAS 1981 Essential -SH groups of photosynthetic ($C_4$) PEP carboxylase are protected by substrate (PEP) and allosteric activator (glucose-6-phosphate). *In* G Akoyunoglou, ed, Photosynthesis, Vol 4. Balaban International Science Services, Philadelphia, pp 99–105
18. 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
19. MANETAS Y, NA GAVALAS 1983 Reduced glutathione as an effector of phos-

phoenolpyruvate carboxylase of the Crassulacean Acid Metabolism plant *Sedum praealtum* D.C. Plant Physiol 71: 187–189
20. MIYACHI S, A KAMIYA, S MIYACHI 1980 Effects of blue light on respiration and non-photosynthetic $CO_2$ fixation in *Chlorella vulgaris* 11 h cells. *In* H Senger, ed, The Blue Light Syndrome. Springer-Verlag, Berlin, pp 321–328
21. MUKERJI SK 1977 Corn leaf phosphoenolpyruvate carboxylases. Purification and properties of two isoenzymes. Arch Biochem Biophys 182: 343–351
22. OLESEN P 1974 Leaf anatomy and ultrastructure of chloroplasts in *Salsola kali* L. as related to the $C_4$-pathway of photosynthesis. Bot Notiser 127: 352–363
23. PERROT C, J VIDAL, A BURLET, P GADAL1981 On the cellular localization of phosphoenolpyruvate carboxylase. Planta 151: 226–231
24. SHOMER-ILAN A, R NEUMANN-GANMORE, Y WAISEL 1979 Biochemical specialization of photosynthetic cell layers and carbon flow paths in *Suaeda monoica*. Plant Physiol 64: 963–965
25. SHOMER-ILAN A, A NISSENBAUM, Y WAISEL 1981 Photosynthetic pathways and the ecological distribution of the Chenopodiaceae in Israel. Oecologia 48: 244–248
26. SLACK CR 1968 The photoactivation of a phosphopyruvate synthase in leaves of *Amaranthus palmeri*. Biochem Biophys Res Commun 30: 483–488
27. UEDAN K, T SUGIYAMA 1976 Purification and characterization of phosphoenolpyruvate carboxylase from maize leaves. Plant Physiol 57: 906–910
28. VON WILLERT DJ, E BRINCKMANN, B SCHEITLER, DA THOMAS, S TREICHEL 1979 The activity and malate inhibition/stimulation of PEP-carboxylase in Crassulacean Acid Metabolism plants in their natural environment. Planta 147: 31–36
29. WINTER K 1980 Day/night changes in the sensitivity of PEP-carboxylase to malate during Crassulacean Acid Metabolism. Plant Physiol 65: 792–796
30. WOLOSIUK RA, BB BUCHANAN 1978 Activation of chloroplast NADP-linked glyceraldehyde-3-phosphate dehydrogenase by the ferredoxin/thioredoxin system. Plant Physiol 61: 669–671