# THE PHOTOACTIVATION OF A PHOSPHOPYRUVATE SYNTHASE IN LEAVES OF AMARANTHUS PALMERI

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One of the reactions of a pathway of photosynthetic $CO_2$ fixation operative in sugar cane (Hatch and Slack, 1966), other tropical grasses (Hatch, Slack and Johnson, 1967) and in members of the Amaranthaceae (Johnson and Hatch, 1968) is the conversion of pyruvate to phosphopyruvate. A phosphopyruvate (PEP) synthase, which catalyses the following overall reaction

$$\text{pyruvate} + \text{ATP} + \text{Pi} \rightleftharpoons \text{phosphopyruvate} + \text{AMP} + \text{PPi}$$

has been partially purified from sugar cane leaves and identified in other tropical grasses (Hatch and Slack, 1968) and Amaranthus species (Johnson and Hatch, 1968). The equilibrium of the reaction favours pyruvate formation. However, we have suggested (Hatch and Slack, 1968) that the operation of PEP carboxylase, adenylate kinase and PPase, which are found in large amounts in the leaves of species that contain PEP synthase, would act to displace the reaction to the right.

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Abbreviations used - DTT, dithiothreitol; RDP, ribulose diphosphate; PEP, phosphopyruvate, PP, pyrophosphate.

The present communication reports that light is required for the maintenance of PEP synthase activity within the leaf. Activity declined when plants were placed in darkness in a manner characteristic of a first order reaction; half-time for loss of activity was 15 mins. When plants were transferred from darkness to full sunlight there was an immediate rapid increase in activity.

## MATERIALS AND METHODS

Plants of Amaranthus palmeri were grown in vermiculite culture at 29° in full sunlight. Daylength was 12 hr. Treatment was provided by placing plants at 29° in a darkened controlled temperature room. At harvest 4 fully expanded leaves (approximately 2 g) were ground with sand in 0.1M tris-HCl buffer (pH 8.3) containing 5mM DTT and 5mM $MgCl_2$ at 0°. The homogenate was filtered through miracloth (Chicopee Mills, Inc. New York), the filtrate (1.0ml) applied to a column of Sephadex G-25 (12 x 0.8 cm), previously equilibrated with 0.05M tris-HCl buffer (pH 8.3) containing 5mM DTT and 5mM $MgCl_2$, and protein collected in 1.5 ml. RDP carboxylase, PEP carboxylase, PEP synthase and adenylate kinase were assayed approximately 10 mins after removing leaves from plants by methods described previously (Slack and Hatch, 1967; Hatch and Slack, 1968). Chlorophyll was estimated in the filtrate by the method of Arnon (1949).

## RESULTS

The activity of PEP synthase in leaf extracts from plants

held in darkness for 24 hr was very much less than in extracts prepared from plants which had been illuminated prior to harvest (Table 1). There was no affect of the dark treatment on the activity of either RDP carboxylase or adenylate kinase but PEP carboxylase activity was 45% less in the dark treated plants. No change in chlorophyll content per unit leaf weight was detected during the 24 hr dark treatment hence, enzyme activities have been expressed per unit weight of chlorophyll to compensate for variability in amounts of cell breakage.

Similar marked reductions in PEP synthase activity have been found in sorghum and sugar cane after a 24 hr dark treatment.

TABLE 1

Activity of PEP synthase and other photosynthetic enzymes in leaves of normally illuminated and dark treated plants.

| Treatment | Enzyme Activity μmole/mg chlorophyll/min. | | | |
|---|---|---|---|---|
| | RDP carboxylase | PEP carboxylase | PEP synthase | Adenylate kinase |
| Normal Illumination | 1.4 | 13.2 | 1.8 | 33.0 |
| 24 hr darkness | 1.6 | 7.5 | 0.15 | 35.0 |

Harvests were made at 3.00pm. Normally illuminated plants had received sunlight from 6.00am. Plants given dark treatment were placed in darkness at 3.00pm the previous day.

More detailed information about the loss of PEP synthase activity after transferring plants to darkness is shown in Figure I. The time-course for loss of activity was characteristic of a

first order reaction having a half-time for loss of about 15 min.

During 50 min in darkness the PEP synthase activity was reduced

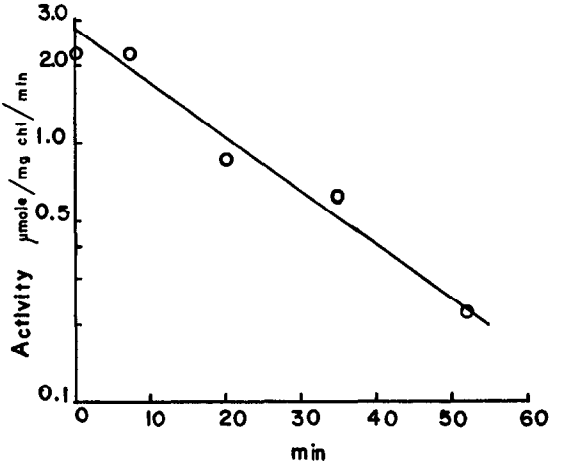


Figure 1 - Decline in phosphopyruvate synthase activity on transferring light-grown plants to darkness. Plants previously grown in a glass house were transferred to a darkened growth room. At intervals leaves were removed in darkness, wrapped in foil and transferred to the laboratory. The time intervals shown are from transfer to darkness to homogenising the leaf.

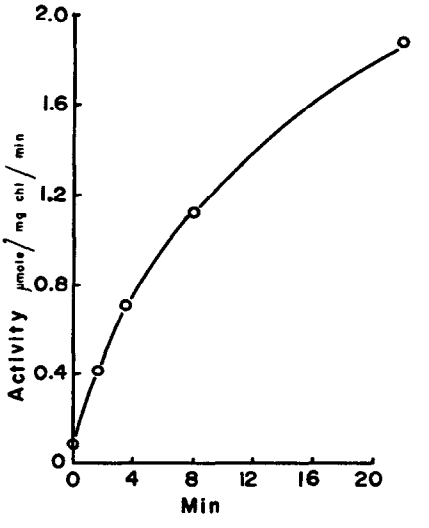


Figure 2 - Increase in phosphopyruvate synthase activity on transferring dark-treated plants to sunlight. Plants kept for 4 hr in darkness were transferred to full sunlight and leaves harvested at intervals thereafter. Homogenisation and treatment on Sephadex was carried out in full sunlight.

to about one-tenth that in the light, over this same period PEP carboxylase showed a 15% loss of activity.

The increase in phosphopyruvate synthase after transferring dark-treated plants to light is shown in Figure 2. During the 22 min period after transfer to light activity increased 25-fold to a value similar to that of plants which had received no dark-treatment.

## DISCUSSION

The increase in activity observed on transferring darkened plants to light may represent either the reactivation of inactivated enzyme or de-novo enzyme synthesis. If light causes the production of an inducer of PEP synthase synthesis a lag period would be expected after transfer to light before the appearance of activity such as is observed after the induction of β-galactosidase (Pardee and Prestige, 1961). However, on transfer to light the maximum rate of increase of enzyme activity occurred during the first $1\frac{1}{2}$ min, and the total activity increased 25-fold in 22 min. These observations suggest that the increase represents activation rather than enzyme synthesis.

Non-aqueous chloroplast isolation from Amaranthus and maize leaf indicates that PEP synthase is localised in the chloroplast (unpublished observation). We have shown that the enzyme is a sulphydryl protein which is irreversibly inactivated in solution in the absence of a -SH compound (Hatch and Slack, 1968).

Consequently, the inactivation by darkness and reactivation on transfer to light may reflect the changing concentration of some reductant in the chloroplast. PEP synthase differs from the photoactivated ATPase described by Petrack and Lipmann (1960) which is activated in solution by -SH compounds. However, some increase in activity in homogenates prepared from dark-treated leaves has been obtained by incubating the homogenates with chloroplast particles in the light.

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