Plant Physiol. (1990) 94, 384–387
0032-0889/90/94/0384/04/$01.00/0

Received for publication May 24, 1990
Accepted June 25, 1990

Communication

Light Induction and the Effect of Nitrogen Status upon the Activity of Carbonic Anhydrase in Maize Leaves[1]

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ABSTRACT

The regulation of carbonic anhydrase (CA) activity in maize (*Zea mays* L.) leaves by light and nitrogen nutrition was determined. CA activity increased by more than 100-fold in illuminated leaves and decreased in leaves placed in the dark; low levels of CA activity were observed in leaves illuminated with low light intensities. CA activity was reduced in plants grown under nitrogen deficiency and recovered only slowly when supplemented with nitrate. Parallel studies were conducted to follow the levels of phospho*enol*pyruvate carboxylase. Experiments indicate that the level of CA and phospho*enol*pyruvate carboxylase present in leaves may be controlled by similar mechanisms.

The recent recognition of the importance of CA[2] (carbonate dehydratase EC 4.2.1.1) to C_4 photosynthesis in catalyzing the hydration of CO_2 to HCO_3^-, the substrate of PEP carboxylase (4, 8, 10), and the report that CA is present in levels of activity only just high enough to satisfy the observed rates of photosynthesis, raises the possibility that, under certain environmental conditions, CA may limit the rate of C_4 photosynthesis. Although there are recent reports in the literature concerning the regulation of expression of CA in unicellular algae, there has been scant regard given to studying the environmental factors which regulate the levels of CA activity in plants, and, in particular, C_4 plants. In this paper we report on the effect of light and N supply on the level of activity of CA in maize leaves. From these studies, together with previous findings that CA levels may be close to rate limiting (8), we conclude that CA levels in plants may, under certain conditions, be growth limiting, and that the control of expression of CA may be similar to that controlling the expression of the PEP carboxylase gene.

MATERIALS AND METHODS

Plant Material

Maize (*Zea mays* L. cv Golden Cross Bantam T51) plants were grown in a growth chamber in vermiculite initially moistened with water and then supplemented with nutrient solution as described previously (15) with N-deficient nutrient mix containing 0.8 mM nitrate and the N-sufficient nutrient mix containing 16 mM nitrate.

Light/Dark Experiments

Plants, initially germinated and grown in complete darkness for 6 d at 22°C, were provided with N-sufficient media. On d 7 plant trays were moved to an artificially illuminated growth chamber (quantum flux of 40 μmol m^{-2} s^{-1}), and after a further 5 d plant trays were moved to a growth chamber illuminated with artificial light with a higher quantum flux (425 μmol m^{-2} s^{-1}). Leaf samples, taken from plants immediately above the leaf sheath, were weighed, sliced finely with a razor blade, and ground in a precooled mortar in 2 mL (g fresh weight)$^{-1}$ of leaf material of extraction buffer (50 mM Hepes-KOH, 10 mM $MgSO_4$, 1 mM EDTA, and 5 mM DTT). The homogenate was filtered through two layers of Miracloth (Calbiochem), a sample removed for Chl determination, and the filtrate centrifuged at 15,000 rpm for 5 min at 0°C. Enzyme determinations were conducted immediately following centrifugation and then 30 min after incubation at 30°C to ensure complete activation of enzymes.

Assays

PEP carboxylase activity was assayed spectrophotometrically (1) and CA activity was assayed colorimetrically (17) as previously described. PEP carboxylase activity is expressed in μmol (min·g fresh weight)$^{-1}$ and CA activity is expressed in Wilbur-Anderson units ($10[T_0/T_E-1]$, where T_0 = time in s required for blue color to be completely lost in reactions containing extraction buffer and TE = time in s required for blue color to disappear completely in the presence of enzyme). Chl was extracted and determined in ethanol as previously described (19).

RESULTS

Initial experiments using leaves from 2 week old maize plants demonstrated that the CA activity varies along the length of a maize leaf with low activity at the leaf base and increasing toward the leaf tip following a pattern similar to

[1] Supported by the Japanese Ministry of Education, Science, and Culture.

[2] Abbreviations CA, carbonic anhydrase; PEP carboxylase, phospho*enol*pyruvate carboxylase.

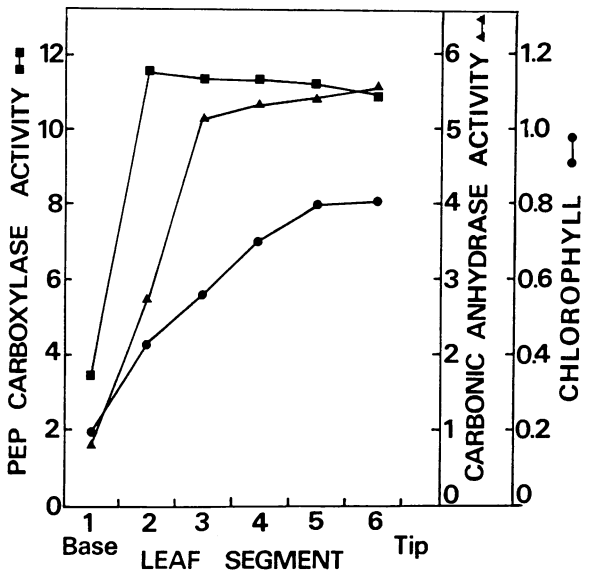

Figure 1. Enzyme activities and Chl concentration as a function of leaf position. Leaves from plants grown in a growth chamber illuminated with artificial light (quantum flux of 425 μmol m^{-2} s^{-1}) 14 h light/10 h dark at 28°C and 20°C, respectively, were divided into six segments of equal length, and CA, PEP carboxylase activities, and Chl concentration in each segment were determined. PEP carboxylase activity is expressed in μmol (min·g fresh weight)$^{-1}$, CA activity is expressed in Wilbur-Anderson units (min·g fresh weight)$^{-1}$ ($\times 10^{-3}$), and Chl content is measured in mg (g fresh weight)$^{-1}$.

the development of both Chl and PEP carboxylase activity along the leaf (Fig. 1). With this knowledge, in experiments to determine the effect of light on the induction of CA activity in leaves we used leaf samples taken from above the leaf sheath to avoid differences in activity along the length of the leaf. In addition, because the Chl concentration in leaves varied in plants grown in the dark and in plants grown on N-deficient media, enzyme activities are expressed in terms of g of fresh weight of leaf material. CA activity was very low in plants grown in the dark; activities were less than 1% of the activities found in the leaves of 2 week old plants grown under full sunlight. CA activity increased rapidly in leaves of plants placed in the light, following a pattern of development similar to that of PEP carboxylase (Fig. 2). The activities of both enzymes increased almost linearly for more than 3 d before reaching a maximum. This activity remained constant while plants were grown under constant light conditions. However, enzyme activities increased when plants were transferred to a growth chamber with higher intensities and decreased in leaves of plants placed in darkness (Fig. 2).

There was no evidence of CA activity being influenced by short term growth conditions; CA activities of plants placed in the dark for 6 h showed no decrease in activity compared with plants grown in high light, indicating that the enzyme was not subject to inactivation in the dark.

The effect of N availability on the activity of CA, PEP carboxylase, and Chl was studied. Leaves of plants grown on limiting amounts of nitrate (0.8 mM) were paler compared with those grown in full sunlight and this was reflected in the lower Chl content of the leaves. Both the CA and PEP carboxylase activities were lower in the N-deficient plants compared to plants grown under N-sufficient conditions. Chl levels and CA and PEP carboxylase activities increased only slowly in plants moved from N-deficient to N-sufficient conditions (Fig. 3).

DISCUSSION

In the leaves of graminaceous plants there is a developmental gradient with young undifferentiated cells at the leaf base and more differentiated cells at the leaf tip. This developmental gradient has been exploited to investigate particular aspects of leaf development in a variety of C_3 and C_4 plants (2, 5, 13, 16, 18) but these studies have not included CA. More recently this gradient has been used to study the level of mRNA and the amount of several proteins involved in photosynthesis in C_4 plants (12, 15). In our present studies total CA activity was very low at the leaf base and increased toward the leaf tip; the total PEP carboxylase activity also increased with distance from the leaf base and closely followed the increase in CA activity. The increase in PEP carboxylase activity as a function of leaf location was similar to the accumulation of leaf mRNA and protein as a function of leaf location in maize leaves previously reported (12). To avoid

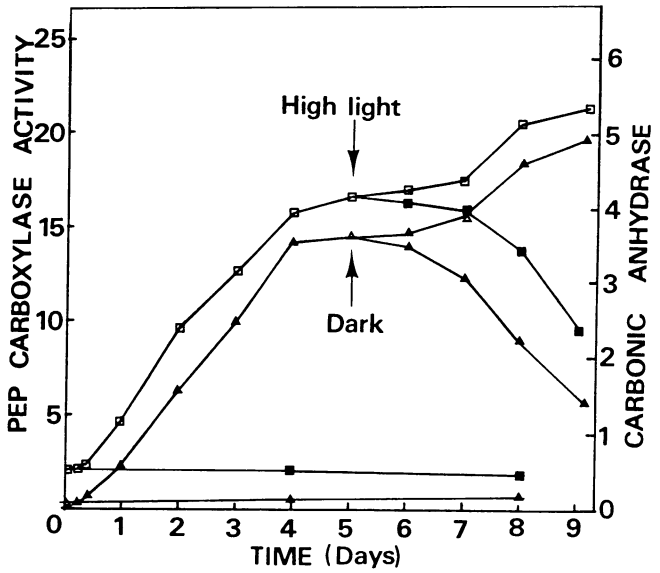

Figure 2. Effect of light intensity on the activity of CA and PEP carboxylase. Maize plants were germinated and grown in complete darkness for 7 d at 25°C. Plants were then transferred to a growth chamber continuously illuminated with artificial light (quantum flux of 40 μmol m^{-2} s^{-1}). Dark control plants remained in the dark for the duration of the experiment. After 5 d, plants in the light were transferred to a growth chamber illuminated with artificial light (quantum flux of 425 μmol m^{-2} s^{-1}) with 14 h light/10 h dark at 28°C and 20°C, respectively. Enzyme activities were determined in plants after 5 h of illumination. Squares denote PEP carboxylase activity and triangles denote CA activity. Open symbols denote light and filled symbols denote dark conditions. Enzyme units used are the same as those used in Figure 1.

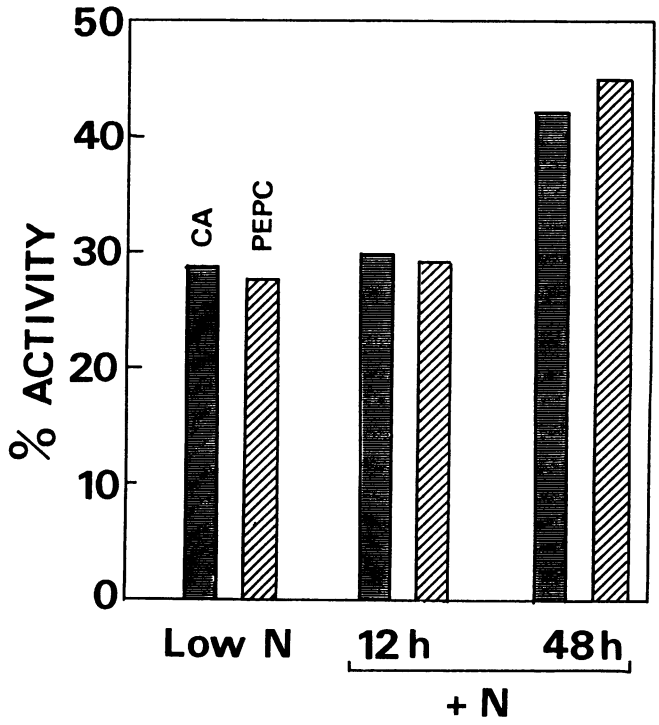

Figure 3. Effect of N nutrition on CA and PEP carboxylase activity in maize leaves. Maize plants were initially grown in N-deficient media (0.8 mM No_3^-) for 2 weeks and then supplemented with NO_3^- (16 mM) in a growth chamber as described in Figure 1. Enzyme levels in plants grown in N-sufficient media (16 mM NO_3^-) were 4,320 and 16.3 units (g fresh weight)$^{-1}$ for CA and PEP carboxylase, respectively. CA activity is denoted by the stippled boxes and PEP carboxylase by the cross-hatched boxes.

variation between samples of leaf tissue taken to determine the effect of both light and N nutrition on the activity of CA and PEP carboxylase, leaves were sampled above the level of the leaf sheath. In contrast to previous findings (7), we found no evidence of CA activity being influenced by short term light conditions. The effect of N nutrition upon the level of PEP carboxylase in C_4 plants has also been determined but no studies have reported the effect of N nutrition on CA (9, 11, 15, 18, 20). With the recognition of the importance of CA in the C_4 acid pathway, and in light of the recent report that CA activity in the cytosol of mesophyll cells of C_4 plants may be only just sufficient to satisfy the requirements for the observed rates of C_4 photosynthesis (8), any decrease in CA activity due to environmental factors may ultimately limit the rate of growth of C_4 plants. For example zinc deficiency may have a more dramatic effect on the rate of photosynthesis in C_4 plants compared with the effects reported for C_3 plants (3, 6, 14).

In the case of both the environmental effects reported here, the development of CA activity was very similar to the development of PEP carboxylase activity. This raises the possibility that in C_4 plants, the expression of CA may be controlled by the same (or a very similar) mechanism to that which controls the expression of PEP carboxylase. Genomic clones of PEP carboxylase have been isolated in a number of laboratories and investigations made to determine the regions responsible for the regulation of expression of the PEP carboxylase gene. With the close similarity of the effect of light and N nutrition upon the expression of both CA and PEP carboxylase, and the close biochemical relationship between the two enzymes, expression of the genes coding for the two proteins may be closely related.

ACKNOWLEDGMENT

This study was carried out while J.N.B. was the grateful recipient of a Fellowship from the Japan Society for the Promotion of Science.

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