Short Communication

Photoperiodism and Enzyme Activity: Balance between Inhibition and Induction of the Crassulacean Acid Metabolism

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From the mass of information on photoperiodic control of flower formation (2, 3), it appears that experimental and theoretical evidence supports the hypothesis of a balance, or competition, between two processes occurring in plant leaves which result in the formation of a flowering inhibitor under noninductive conditions and a flowering stimulus under inductive conditions. Particularly in reference to Kalanchoe blossfeldiana, a short day plant, Schwabe (10, 11) described some results consistent with the presence of an inhibitor of flowering in crude sap expressed from Kalanchoe leaves grown under noninductive conditions. We report here that this model for a dual action of photoperiodism can be extended to the control of an enzymatic pathway, and hence the action may be understood at the metabolic level.

The phrase CAM1 defines a metabolic pathway which includes, (a) the synthesis of malate, through the coupled operation of P-enolpyruvate carboxylase EC 4.1.1.31 and malate dehydrogenase EC 1.1.1.37; (b) the decarboxylation of malate by malic enzyme EC 1.1.1.40; and (c) the transamination reaction between oxaloacetate and glutamate catalyzed by aspartate aminotransferase EC 2.5.1.1. In young leaves of Kalanchoe blossfeldiana Tom Thumb, the activity of these enzymes is under photoperiodic and phytochrome control (8, 9). In long days or in short days with nights interrupted by red light, the pathway is not operative presumably due to the activity of P-enolpyruvate carboxylase being very small. In short days, there is a progressively rapid increase in the activity of all the enzymes of the pathway and a net accumulation of malate.

Acid accumulation starts after a lag period of about 7 short days. It has been suggested that this lag time would correspond with a residual long day effect (7). In fact, Morel et al. (5) have shown that total activity of P-enolpyruvate carboxylase increases following two successive exponential functions and that the change in slope takes place after about 30 short days. We propose an interpretation according to which, (a) the 7th short day is a threshold for P-enolpyruvate carboxylase activity, which then becomes detectable in vitro and efficient in vivo, as shown by the changes in the amounts of malate; (b) the 30th short day would mark a critical low level of an inhibitory system produced in long days, the disappearance of this inhibitor progressively unmasking the increase in P-enolpyruvate carboxylase. Our results confirm these hypotheses.

1 Abbreviation: CAM: Crassulacean acid metabolism.

MATERIALS AND METHODS

Kalanchoe blossfeldiana Tom Thumb was cultivated in the phytotron of Gif-sur-Yvette under noninductive long days of 16 hr (mixed fluorescent Lumiere du Jour de Luxe 125 w and incandescent light, 100,000 ergs cm−2sec−1). After 2 months, the plants were given inductive short days (9-hr day) or noninductive control treatment (9-hr day followed by interruption of the dark period with a single exposure to 250 ergs cm−2sec−1 red light from red fluorescent tubes Philips 40W TL15).

Crude extracts of young leaves (second pair from the apex) were prepared by rapid and thorough grinding in 0.2 M tris-HCl buffer, final pH 7.4, containing 3.5 mM MgCl2 and with or without given amounts of polyethylene glycol molecular weight 20,000 (from Touzart et Matignon, Paris) as indicated in “Results and Discussion.” Total enzyme activity was measured in the supernatant from high speed centrifugation, by spectrophotometric methods, in 0.2 M tris-HCl buffer, pH 7.4, at 30 C. P-enolpyruvate carboxylase was assayed by coupling with the excess malate dehydrogenase activity present in leaf extracts. The assay contained, in 3 ml, 10 μmoles MgCl2, 5 μmoles NaF, 7 to 20 μmoles P-enolpyruvate, 0.36 μ mole NADH, and enzyme extract corresponding to 0.10 to 0.25 mg dry weight of leaves. Malate dehydrogenase was assayed in 3 ml, with 5 μmoles MgCl2, 3 to 6 μmoles oxaloacetate, 0.36 μ mole NADH, and enzyme extract corresponding to 0.005 to 0.030 mg dry weight of leaves. Malic enzyme assay was carried out in 3 ml with 10 μmoles MgCl2, 20 to 80 μmoles malate, 0.36 μ mole NADP, and extract corresponding to 0.20 to 0.50 mg dry weight. Glucose-6-P dehydrogenase EC 1.1.1.47 was assayed in 3 ml containing 5 μmoles MgCl2, 10 μmoles glucose-6-P, 0.36 μ mole NADP, and extract corresponding to 0.50 mg dry weight of leaves. In all these assays, the change in absorbance was recorded at 340 nm. Aspartate aminotransferase activity was assayed at 280 nm in 3 ml containing 3 to 9 μmoles oxaloacetate, 20 to 70 μmoles glutamate, and extract corresponding to 0.10 to 0.50 mg dry weight of leaves. The amount of pyridoxal phosphate present in the extract was shown to be sufficient to allow maximum enzyme activity.

RESULTS AND DISCUSSION

Extracts with CAM activity (“active extracts”), can be obtained from plants in short days only if polyethylene glycol is present in the extraction medium (1). In the absence of polyethylene glycol, all the extracts obtained from plants either
in noninductive or even in inductive photoperiod, display no activity of the CAM enzymes. Such extracts have been named "inhibitory extracts," since when added to active extracts they all have, in a greater or lesser degree, the ability to reduce partially or completely the activity of these extracts. Polyethylene glycol is known to prevent or to remove enzyme inactivation (4). The question arises whether the inhibitor has an action in vivo on CAM activity or whether it inactivates the enzymes during extraction. We know that a limited amount of CAM enzymes is present in leaves grown in noninductive conditions, as demonstrated by activation assays in vitro (1) and by extraction with high amounts of polyethylene glycol (higher than 2.5% w/w of the fresh weight of leaves). Nevertheless, these enzymes have no activity in vivo under noninductive conditions as shown by the low amount of malate in the leaves and by the absence of dark CO₂ fixation (6, 7). The following data are supporting evidence that these enzymes are in an inhibited form in vivo under long days or under short days with interrupted night.

The control by photoperiodism of the amount of inhibitor present in leaves is shown in Figure 1a, where the action of inhibitory extracts when added to pig heart malate dehydrogenase is expressed by the loss in enzyme activity. After a 7 short day lag period, the amount of inhibitor drops rapidly up to the 30th short day and then stabilizes at a very low but irreducible level. Such behavior agrees with what would be expected from the kinetics of P-enolpyruvate carboxylase increase and malate production by the leaves (Fig. 1b) if the inhibitor acts on CAM enzymes in vivo. The effective role of phytochrome in the inhibitor production is clearly established by the difference between the curves in Figure 1a: in leaves grown under short days with interruption of night by red light, the inhibitory effect of the extracts remains continuously high; under inductive short days, the inhibitory capacity stabilizes at a finally low level, about 5% of the initial value. As the remainder amount of inhibitor cannot prevent in vivo CAM activity in short days, the hypothesis of compartmentation must be considered: only the pool of inhibitor in contact with CAM enzymes would be controlled by photoperiodism.

In order to verify the connection between this inhibitor and the activity of the CAM pathway, we tested the specific action of inhibitory extracts from plants treated with night interruption, on active extracts (polyethylene glycol 2% w/w of fresh weight of leaves) from plants in short days (Fig. 1c) and found that the enzymes of the pathway (P-enolpyruvate carboxylase, malate dehydrogenase, aspartate aminotransferase) appear to be inhibited at different levels. An important result is that the inhibitory system has no action, even at high concentration, on malic enzyme; the same holds true concerning glucose-6-P dehydrogenase, an enzyme which is presumably not connected with CAM.

The characteristics of the inhibitor are under investigation, at present, and it has been shown to be dialysable and thermostable and to have at least some properties of a tannin-type substance. Noncompetitive inhibition with regard to substrate has been demonstrated in assays with pig heart malate dehydrogenase and with P-enolpyruvate carboxylase and aspartate aminotransferase from dialysed plant extracts.

To sum up, our initial hypothesis is supported by the data presented in this paper: an inhibitory system is produced by young leaves in long days and disappears in short days. This system has some degree of specificity for CAM enzymes, which are under the control of photoperiodism and phytochrome in these plants.

Hence, it appears that the change from long days to short days sets into motion two processes: arrest of production of an inhibitor, which then disappears progressively and induction of large amount of CAM enzymes, mainly P-enolpyruvate carboxylase. The observed kinetics of both processes can be considered as the result of a balance between inhibitor and enzymic activities. This double mechanism appears to be similar to that described by Schwabe (10) from his results on flowering. In the case of Kalanchoe blossfeldiana, induction time for flowering is 7 short days under the present
experimental conditions, and this is also the lag time for the start of malate accumulation.

It is noteworthy that two "terminal" phenomena (flowering and malate accumulation) belonging to different levels of physiological organization (morphogenetic and metabolic) display similar characteristics. This would support the unitary hypothesis that one and the same type of mechanism would account for the first steps of different photoperiodic-controlled processes.

**LITERATURE CITED**


