

Development of Ribulose-1,5-Diphosphate Carboxylase in Castor Bean Cotyledons

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ABSTRACT

Light was not essential for the development of ribulose-1,5-diphosphate carboxylase protein or catalytic activity in the photosynthetic cotyledons of germinating castor beans (*Ricinus communis*). Cotyledons developing in the dark showed higher activity than those in the light. Returning cotyledons developing in the light to darkness resulted in a significant increase in ribulose-1,5-diphosphate carboxylase activity compared to cotyledons in continuous light.

The illumination of dark-grown bleached *Euglena gracilis* cells initiates the synthesis of the chloroplast enzyme ribulose-1,5-diphosphate carboxylase¹ (17). The activity of this enzyme increases along with other enzymic and structural components of the developing chloroplast, for 3 to 4 days, by which time the cells are photosynthetically competent (4, 7). If at any time during the regreening process *Euglena* cells are transferred back into darkness there is a striking continued synthesis of RuDP Case in the dark (10, 11). As with *Euglena*, light initiates the synthesis of RuDP Case in dark-grown etiolated higher plant tissues (9, 13), but the effect of subsequent darkening in RuDP Case activity has not been investigated. The present study was concerned with the effect of intermittent illumination on RuDP Case activity in a greening higher plant tissue, castor bean cotyledons.

MATERIALS AND METHODS

Plant Material. Seeds of castor bean (*Ricinus communis*) were soaked overnight in running tap water and were grown in moist vermiculite at 30 C; 4-day-old seedlings were chosen for uniformity and planted in pots of John Innes No. 1 Compost, all manipulations being carried out under a green safelight in a darkroom. Plants were kept at 25 C in the dark until the endosperm had softened and split when plants were illuminated by warm-white (Osram) fluorescent tubes at an intensity of 10,000 lux. Control plants were kept in continuous darkness, and at intervals some illuminated plants were returned to the dark.

Homogenization. Cotyledons were homogenized in a medium containing 150 mM Tricine (pH 7.5), 10 mM KCl, 1 mM EDTA, 1 mM MgCl₂, 0.4 mM sucrose by grinding with sand in a cooled mortar for 5 min. The homogenate was centrifuged at 500 g for 10 min and the supernatant used for assays. Endosperm halves were homogenized by grinding with the same medium but without sand for 5 min in a mortar, the homogenate squeezed

through four layers of cheesecloth, and the crude extract used for assays.

Separation of Cellular Organelles. 20 cotyledon halves from 5-day-old dark-grown seedlings were homogenized by chopping for 5 min with a single razor blade in a Petri dish on ice containing 5 ml of the enzyme extraction medium. The crude homogenate was filtered through four layers of cheesecloth, the residue was extracted with a further 3 ml of grinding medium, and the volume of the filtered homogenate was adjusted to 10 ml with grinding medium. Portions (5-ml) of crude homogenate were layered onto sucrose gradients, prepared as described previously (6), increasing linearly in concentration from 0.5 M to 2.5 M. All sucrose solutions contained 1 mM EDTA (pH 7.5). Gradients were centrifuged for 3 hr at 20,000 rpm (53,000g av.) in a SW 27 rotor in a Beckman L2-65B ultracentrifuge. After centrifugation, 1-ml fractions were collected using a Beckman gradient fractionator.

Enzyme Assays. RuDP Case and fumarase were assayed as described previously (6, 11) NADH-triosephosphate isomerase by the method of Beisenherz (2).

Determination of RuDP Case Protein. Antisera raised against purified *Euglena* and wheat RuDP Case were used for the determination of RuDP Case protein. Quantitative precipitin

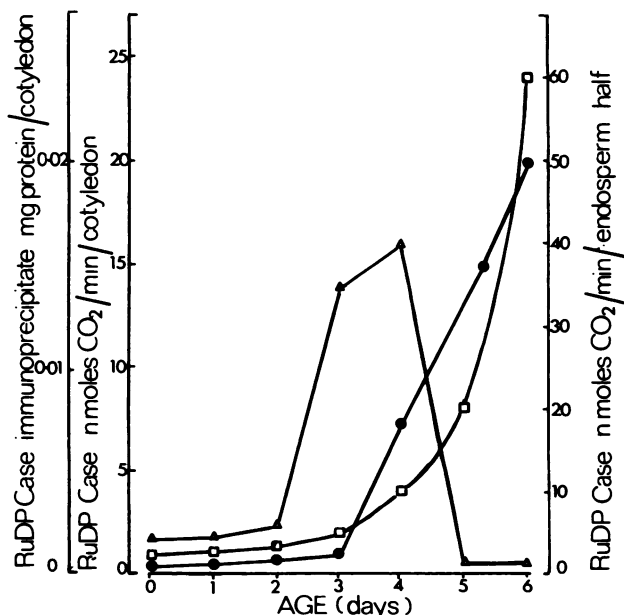


FIG. 1. RuDP Case activity in cotyledons and endosperm during the early stages of castor bean development in the dark. Δ : RuDP Case/endosperm half; \bullet : RuDP Case/cotyledon; \square : RuDP Case immunoprecipitate/cotyledon.

¹ Abbreviation: RuDP Case: ribulose-1,5-diphosphate carboxylase.

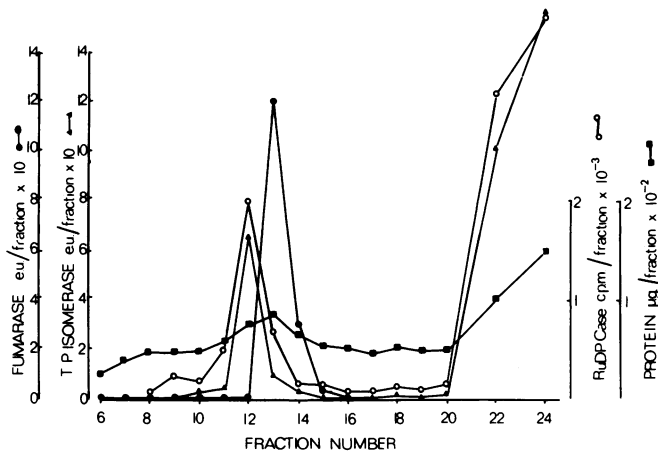


FIG. 2. Equilibrium density gradient centrifugation of organelles from whole homogenate of 5-day cotyledons of dark-grown germinating castor beans. All results are expressed as units where 1 unit is $1 \mu\text{mol}$ of substrate/hr · fraction.

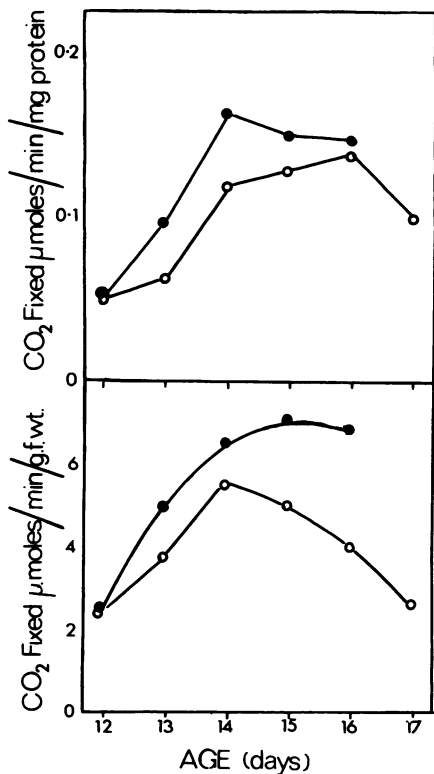


FIG. 3. Development of RuDP Case activity in cotyledons of germinating castor beans in the dark (●) or light (○).

reactions between antigen and antisera were carried out as described previously (5, 15), identical values for castor bean RuDP Case immunoprecipitates being obtained with each antiserum.

Other Methods. Protein was determined by the method of Lowry *et al.* (12), with BSA as standard. Sucrose solutions were determined refractometrically and Chl by the method of Arnon (1).

RESULTS AND DISCUSSION

The development of RuDP Case in the endosperm and cotyledons of germinating castor beans kept in the dark is shown in Figure 1. The development of RuDP Case activity in the endo-

sperm was substantially similar to that described by Benedict (3, 8) and Osmond *et al.* (16). However, as RuDP Case activity declined in the endosperm there was a rapid increase in activity in the cotyledons (Fig. 1) reaching a maximum by day 15 (see Fig. 3). RuDP Case activity in the cotyledon was barely detectable on day 3 being less than $1 \text{ nmol/min/mg protein}$ but by day 15 this had increased to $170 \text{ nmol/min/mg protein}$. This substantial increase in catalytic activity during cotyledon development was accompanied by a parallel increase in RuDP Case protein (Fig. 1) showing that the increase in catalytic activity was the result of *de novo* synthesis of protein. The final level of RuDP Case activity in dark-grown cotyledons is similar to that in photosynthetically competent tissues; for comparison, the specific activity of RuDP Case in crude homogenates of *Euglena* was $127 \text{ nmol/min/mg protein}$ (14).

The centrifugation of whole homogenates of 5-day-old cotyledons on sucrose gradients showed that some RuDP Case activity was associated with the proplastid fraction (Fig. 2). A peak of RuDP Case activity at a density of 1.23 g/cm^3 coincided with the peak of triosephosphate isomerase activity, a marker enzyme for proplastids (Fig. 2). Surprisingly, the development of RuDP Case activity in castor bean cotyledons was not light-dependent (Fig. 3), maximal levels of RuDP Case being recorded in cotyledons maintained in continuous darkness (Fig. 3). If cotyledons were illuminated during development, RuDP Case activity expressed on either a protein or fresh weight basis was lower than that in dark-grown cotyledons (Fig. 3).

When cotyledons at different stages of development in the light were returned to the dark for 24 and 48 hr, these cotyledons showed far higher levels of RuDP Case activity than comparable cotyledons kept continuously in the light (Fig. 4). It could be argued that returning cotyledons to the dark decreased fresh weight and protein so giving apparently higher RuDP Case levels. However, returning cotyledons to the dark halted Chl synthesis so that after 24 hr in darkness the Chl content of

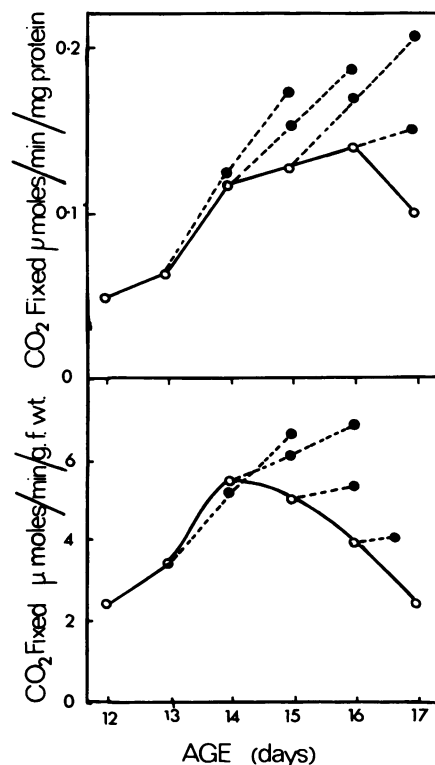


FIG. 4. Development of RuDP Case activity in cotyledons of germinating castor beans in the light (○) and after transferring cotyledons from the light to darkness (●).

cotyledons was unchanged (Fig. 5). Expressing RuDP Case activity on a Chl basis still revealed a striking increase in activity on returning cotyledons from light to the dark (Fig. 5).

CONCLUSIONS

Light was neither necessary to initiate RuDP Case synthesis in castor bean cotyledons, nor required for the continuing synthesis of the enzyme, dark-grown cotyledons showing greater RuDP

Case activity than cotyledons kept in the light. In contrast, light was required to initiate RuDP Case synthesis in dark-grown etiolated leaves (9, 13) and dark-grown *Euglena* (17). When dark-grown cultures of *E. gracilis* regreening in the light were transferred back into darkness, there was a dramatic increase in RuDP Case activity (10, 11) and the same phenomenon was found when castor bean cotyledons developing in the light were put into the dark. As far as we are aware, castor bean cotyledons are the first recorded photosynthetic higher plant tissue in which maximum development of RuDP Case activity occurs in the dark and in which there is a partial repression of RuDP Case activity by light.

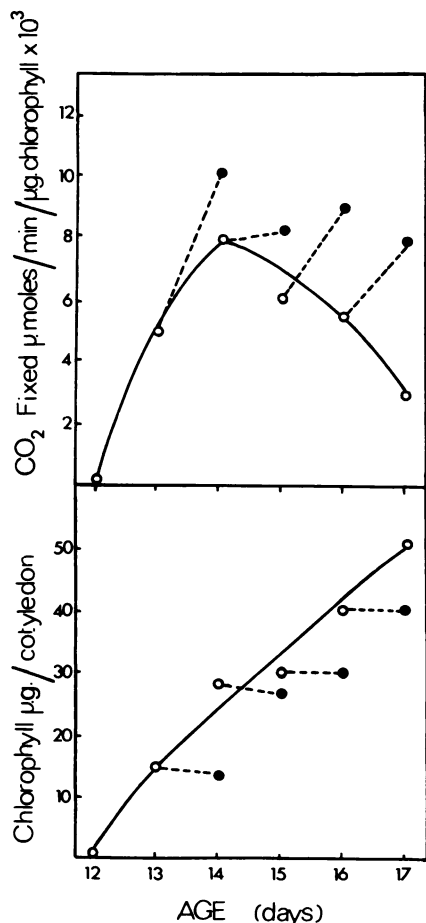


FIG. 5. Chl and RuDP Case activity in cotyledons of germinating castor beans in the light (○) and after transferring cotyledons from the light to darkness (●).

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