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PHOTOCHLOROPHYLLIDE (P650) TURNOVER IN DARK-GROWN BARLEY LEAVES

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; protochlorophyllide (P650); turnover; laevulinate.

Abstract—Laevulinate (LA) induced an increase in protochlorophyllide (P650) in dark-grown ageing barley leaves. The increase was due to a suppression of a P650 breakdown mechanism. The LA inhibition of P650 destruction allowed an estimate to be made of turnover of P650 in ageing etiolated leaves. The rate constant for P650 destruction in 8-day-old dark-grown leaves was 139 pmol/nmol/hr with a half life of 5 hr.

INTRODUCTION

Dark-grown leaves of Angiosperms contain small quantities of protochlorophyllide (P650) [1, 2] which is converted to chlorophyllide [3] and finally to chlorophyll [4] on exposure to light. In light grown tissue P650 is converted almost immediately to chlorophyll. The turnover time for P650 formation is rapid and has been reported as 2.5 min in 6-day-old barley leaves [5]. It is not clear however, whether this is the half life of P650 itself or of one of its precursors. The formation of P650 from P635 takes 20–50 sec in bean leaves, the subsequent photoconversion of chlorophyllide (P678) taking about 1 min [6]. This suggests that the half-life of P650 in the light is less than 60 sec.

P650 concentrations decline with age in etiolated barley leaves [7] because of a rapid fall-off between days 6 and 8 of an aminolevulinic acid (ALA) synthesising system [8]. Experiments were designed to measure the turnover of accumulated porphyrins in dark- and light-treated leaves of barley [9]. The present report deals with the turnover of photoconvertible protochlorophyllide in dark-grown leaves.

RESULTS AND DISCUSSION

Seedlings were grown in the dark for 7, 8 and 10 days. The primary leaves were cut 6 cm below the tip and placed in vials containing laevulinic acid (LA) at 0.1 M, pH 7.5. Control leaves were fed phosphate buffer only (0.5 M, pH 7.5). After 7 hr in the dark, photoconvertible protochlorophyllide (P650) was extracted and determined. The results are given in Table 1. The amount of P650 was at its highest in 8-day-old material and declined by over 33% in 10-day-old leaves.

LA had the unexpected effect of increasing P650 at all ages but particularly in the 8-day-old leaves (by 87.5%).

Table 1. The effect of laevulinic acid (LA) on protochlorophyllide (P650) in dark-grown barley leaves.

Age (days)	Protochlorophyllide (nmol/g fr. wt)			% Promotion
	Controls	LA treatment	Increase	
7	12.0	14.7	2.68	22.4
8	12.3	23.1	10.8	87.5
10	8.82	10.7	1.89	21.4

Seedlings were grown in the dark for 7, 8 and 10 days. The primary leaves at each age were cut 6 cm below the tips and placed in vials containing LA (0.1M, pH 7.5). After 7 hr the leaves were illuminated for 3 min and P650 assayed as its photoconverted form.

As a competitive inhibitor of ALA-dehydratase [10] LA would inhibit, although probably not completely, the formation of porphobilinogen and therefore the synthesis of P650. As P650 increases quite significantly in the presence of LA, the increase cannot be due to a promotion of synthesis. It seems likely, therefore, to be due to a suppression of P650 break-down.

If the amount of LA-induced increase in P650 (Table 1) represents the amount of P650 destroyed in the controls (A), then:

$$A = P_{LA} - P_{CON}\,(\text{after n hr feeding}) \qquad (1)$$

where P_{CON} is the amount of P650 in the cut controls and P_{LA} the amount in the LA treated tissue. Having calculated A, then the amount of P650 *gross* synthesis (B) can be calculated from:-

$$B = P_{t7} - P_{to} + A \qquad (2)$$

Where P_{t7} is the amount of P650 in the cut controls after 7 hr LA feeding and P_{to} the amount at the start of feeding. Values for B in nmol at leaf ages of 7, 8 and 10 days are 0.45, 13.2 and 0.86 respectively.

The rate constants (K) for A and B were calculated from the average pool size (Equation 3) and are given in Table 2.

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Table 2. Rate constants (K) for P650 destruction (A) and synthesis (B)

Leaf age (days)	K(A) (pmol/nmol/hr)	K(B) (pmol/nmol/hr)
7	29.2	4.91
8	138.6	169
10	28.9	13.2

K(A) and K(B) were calculated using equation 3.

$$K = \frac{A \text{ (or B)}}{t} \times \frac{1}{\frac{P_{t7} + P_{to}}{2}} \quad (3)$$

Taking the lower of the two rate constants [13], the half life of P650 can be calculated from Equation 4.

$$t_{\frac{1}{2}} = \log_e 2/K \quad (4)$$

The half-life values for 7-, 8-, and 10-day-old leaves are 141.2, 5 and 52.7 hr respectively.

The results demonstrate that the turnover of P650 is rapid in 8-day-old leaves (and 9-day-old tissue [11]). The rate of P650 synthesis increased 35-fold between days 7 and 8 and thereafter declined.

There are several criticisms which are pertinent. (1) The rate constants show that the P650 pool was not in a steady state. This means that the half-life values for 7- and 10-day-old material are inaccurate. (2) The assumption was made that LA at 0.1 M completely inhibited ALA-dehydrogenase activity and therefore P650 synthesis. This is unlikely to be true. However, if the value of A is underestimated then B would also be underestimated by a similar amount. If this underestimate could be corrected for, it would increase the rate constants and reduce the length of the half-life. As a guide, during the early linear phase of chlorophyll synthesis, LA at 0.1 M, can inhibit chlorophyll formation by as much as 75%. This means that the rate constants for A and B above are underestimated by 25% and the half-life overstated by a similar amount. The half-life of P650 in 8-day-old dark-grown leaves therefore might be nearer 3.75 hr. (3) The assumption was also made that LA completely inhibited P650 breakdown. If there were only partial inhibition then half-life values would have to be decreased by the amount of P650 destruction not suppressed by LA.

With the above provisos, and emphasising that the results apply to cut and not to intact leaves, photoconvertible P650 turnover in the dark appears to be relatively rapid with a half-life of *ca* 4 hr in 8-day-old leaves. This is the age at which P650 reaches its maximum level in intact leaves [7]. Rapid turnover in fact continues to day 9 but is accompanied by a rapid decline in P650, much of the newly formed P650 apparently being broken down. This seemingly wasteful process ceases at day 10 with the resumption of a relatively longer half-life. The levels of P650 in intact leaves remain steady from day 10 to day 16 [7].

DISCUSSION

Laevulinate (LA) at 0.1 M greatly diminishes the concentrations of chlorophyll and protohaem [11]. This is assumed to be due to the inhibition by LA of ALA dehydratase [10] but it may also involve the stimulation of porphyrin degradation perhaps as a result of ALA accumulation in the presence of LA. In the dark, however, LA induced a substantial increase in photoconvertible P650 possibly due to a suppression of a P650 breakdown mechanism mediated by accumulating ALA. Bogorad [4] suggested that P650-reductase might regulate the formation of ALA and demonstrated a possible stoichiometric relationship between the levels of P650 (or holochrome) and the activity of the ALA synthesising enzyme(s). While this scheme is concerned with the regulation of ALA formation by P650 it may have validity in reverse, that is, ALA concentrations or the rate of ALA synthesis, also regulates P650 turnover.

The P650 pool was not in a steady state over the period examined (7 to 10 days). The maximum rate of synthesis and destruction occurred in 8-day-old leaves. Estimates of the half life showed this to be certainly less than 5 hr in 8-day-old material but extending to over 50 hr by day 10. That is, as the leaf ages in the dark the rate of turnover of P650 declines. The increase in half life values follows the decrease in endogenous ALA formation [8] in barley after 8 days in the dark. This again, may indicate that the rate of ALA formation regulates the rate of turnover of P650.

EXPERIMENTAL

Plant material. Barley seeds (*Hordeum vulgare*, cv Proctor) were imbibed for 16 hr in H_2O, planted in trays of vermiculite and germinated at 25° in the dark.

Photoconvertible protochlorophyll estimation. Leaves were exposed to 3 min illumination before extraction in 80% Me_2CO. The *A* at 672 nm was then measured and related to P650 levels using the extinction coefficients of Jones [12].

Half life and rate constant calculations. These are based on definitions and equations presented by refs. [13 and 14].

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