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THE TURNOVER OF CHLOROPHYLL IN GREENING WHEAT LEAVES

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Abstract—A method for the estimation of chlorophyll turnover in wheat leaves is presented This is based on the inhibition of chlorophyll synthesis by treatment of the cut leaves with laevulinic acid (LA), a competitive inhibitor of δ-aminolaevulinic acid dehydratase The turnover of chlorophyll in young, greening leaves, given short periods of light was a relatively rapid process However, in seedlings exposed to light for longer periods the turnover became progressively slower, and was measured in days rather than hours

INTRODUCTION

Evidence for the turnover of chlorophyll, involving the simultaneous measurement of degradation and synthesis, in higher plants, seems poorly documented There is some evidence which indicates that chlorophyll is broken down slowly, in the dark [1–3] Suzer and Sauer [4] found that cycloheximide inhibited chlorophyll production in partially greened barley leaves and that the level remained stable for at least 19 hr A similar result was reported for the chlorophyll in *Chlorella* treated with cycloheximide [5] High light intensity has been found to induce chlorophyll breakdown, both during and after the lag-phase in chlorophyll formation in bean leaves [6] and *Euglena* [7] The effect may be due to the photobleaching of newly formed chlorophyll [8] and the lag phase can be reduced to a few minutes by illumination in dim light [9] or by brief light flashes [10] Virgin [8] suggested that the lag phase in chlorophyll formation is due to the simultaneous synthesis and breakdown of chlorophyll in the absence of an adequate concentration of carotenoids Akoyonuglou's laboratory [11] has shown that chlorophyll turnover may be confined to the immature developing thylakoids in green leaves deprived of light It is not clear, however, whether chlorophyll turnover occurs in the light during the later, post lag, stages of chlorophyll production

Some measure of chlorophyll turnover, in diatoms [12] and *Chlorella* [13], has been made using radioactive procedures This method has proved, in our hands unsuitable for higher plants particularly during the greening process, when only relatively low levels of chlorophyll are present in the leaves

In order to overcome these difficulties we have used laevulinic acid (LA), a competitive inhibitor of δ-aminolaevulinic acid dehydratase [14] By blocking the synthesis of chlorophyll it is possible to estimate the amount of chlorophyll degraded in a given time This report deals with the calculations necessary to use this approach and the turnover of chlorophyll in the greening leaves of young, dark-grown, seedlings of wheat The technique has been previously demonstrated for chlorophyll [15] and protochlorophyllide [16] in barley seedlings, and a haem protein in mung beans [17] The results presented are for total chlorophyll and no attempt has been made to determine separately the turnover of chlorophyll *a* and chlorophyll *b*

RESULTS

The chlorophyll which accumulates in a leaf in a given time (t_n) is the difference between the amount of chlorophyll destroyed (A) and the amount synthesized (B)

$$Pt_n - Pt_0 = B - A \quad (1)$$

where P is the chlorophyll at time 'n' and at time zero (0)

To calculate the half-life of chlorophyll it is necessary to determine A separately from B Values for A can be obtained according to the following rationale leaves in the light undergo concurrent chlorophyll synthesis and breakdown but if the leaves are detached and placed in a solution of laevulinic acid (LA, an inhibitor of chlorophyll synthesis) the chlorophyll will decrease by an amount equal to the quantity of chlorophyll destroyed However, the inhibition of chlorophyll synthesis by LA is not complete ($B \neq 0$) The chlorophyll which is degraded (A) in the leaf is given in equation 2

$$A = [Pt_0 - Pt_n\,(\mathrm{LA})] + B\,(\mathrm{LA}) \quad (2)$$

where Pt_n (LA) is the chlorophyll in leaves treated with LA for time 'n' and B (LA) is the small quantity of chlorophyll synthesised in the presence of the inhibitor

If A, the amount of chlorophyll broken down in the leaves treated with LA, is the same in the cut control (con) leaves then it is possible to estimate 'gross' chlorophyll synthesis [B (con), equation 3]

$$B\,(\mathrm{con}) = [Pt_n\,(\mathrm{con}) - Pt_0] + A \quad (3)$$

Table 1 gives the results of experiments in which 6-day-

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Table 1 Chlorophyll (nmol/leaf) in greening leaves treated with laevulinic acid (LA)

Light (hr)	After light period[1]	7 hr dark (buffer)[2]	7 hr dark (LA)[3]	7 hr light (buffer)[4]	7 hr light (LA)[5]
2	85±19	73±08	70±05	127±20	49±07
3	126±23	116±17	107±12	148±02	59±04
6	185±19	187±21	188±13	227±14	106±08
12	320±34	300±21	287±16	332±21	227±25
24	426±31	402±35	388±27	447±20	339±39
48	732±141	710±73	684±61	737±47	615±34
60	789±63	764±56	769±20	780±41	721±57

Six-day-old, dark-grown, seedlings were transferred to the light At intervals leaves were excised and placed in buffer or LA in the dark for 7 hr, after which they were given a light treatment for a further 7 hr Chlorophyll was determined at the end of the light period (1), after 7 hr in the dark in buffer (2) or LA (3) and after the final light treatment (4 and 5) The results are the mean of three to five replicates each of 20 leaves with standard deviations

old, dark-grown, seedlings were illuminated for periods up to 60 hr At intervals during the light period the leaves were cut at the base, placed in a solution of LA (0 1 M, pH 7 2) for 7 hr in the dark, and then transferred to the light for a further 7 hr in LA Control leaves were treated similarly except that the LA was replaced by buffer only The chlorophyll in the leaves was determined (i) after the initial light treatment, (ii) at the end of the LA or buffer treatment in the dark and (iii) at the end of the further 7 hr in the light The chlorophyll content of the leaves treated with LA and given a 7 hr light treatment was always less than that found in the control leaves From the data in Table 1 it is possible to calculate the amount of chlorophyll synthesized (B) and the amount which breaks down (A) using equations 2 and 3 given above In order to calculate A it is also necessary to obtain a value for the chlorophyll synthesized in the presence of LA [B(LA)] B(LA) was determined from the chlorophyll in the leaves of dark-grown seedlings, of equivalent age to those given the light period (6 days + x hr light) after being cut at the base and placed in LA in the dark for 7 hr followed by 7 hr in the light The protochlorophyllide in the leaves, prior to the 7 hr light treatment, was also determined and the chlorophyll corrected accordingly The results (Table 2) show that the inhibition of chlorophyll synthesis by LA was not complete and hence these values were used for B(LA) in equation 2 The gross amounts of the chlorophyll broken down (A) and synthesized (B) in the light-treated leaves of 6-day-old dark grown seedlings are given in Table 3 Rate constants for the chlorophyll broken-down (Ak) and synthesized (Bk) were calculated using equation 4 (see Experimental) The results (Table 3), expressed as pmol/nmol chlorophyll/hr, indicate that a steady state in synthesis and breakdown probably exists in the leaves of seedlings given the 6 hr light period before cutting and placing in LA or buffer Light periods of a longer duration result in a rate of synthesis greater than breakdown The turnover of chlorophyll is expressed as half-life, or more correctly, 'half-time' values, in Table 3 The turnover of chlorophyll was between 6 and 8 hr in the leaves of seedlings given a 2–6 hr light period After 12 hr light, however, the turnover of chlorophyll became slower until, in the leaves from seedlings given 60 hr treatment, it was in the order of days

Table 2 Chlorophyll in the leaves of dark-grown seedlings treated with laevulinic acid (LA) and exposed to light

Leaf age	Chlorophyll (nmol/leaf)
6-day + 2 hr	15±04
" + 3 hr	13±04
" + 6 hr	23±09
" + 12 hr	19±05
" + 24 hr	19±04
" + 48 hr	18±06
" + 60 hr	18±04

The leaves of 6-day-old, dark-grown seedlings were excised after a further 2–60 hr of growth and placed in LA for 7 hr in the dark The leaves, in LA, were then given a 7 hr light treatment and the chlorophyll content determined The results are the mean of three replicates each of 20 leaves and are corrected for photoconvertible protochlorophyllide

DISCUSSION

The total chlorophyll in the young leaves of wheat seedlings is continually being 'turned over' in the light The half-time values for chlorophyll in leaves of seedlings given up to a 6 hr light period is relatively rapid However, with increased exposure to light the turnover of chlorophyll takes progressively longer After a 60 hr light-period the chlorophyll in the leaves has a half-time value of about 2–5 days

It should be noted, however, that the true 'half-time' values for chlorophyll are probably slightly shorter than the values presented The difference arises from the fact that the chlorophyll degraded in the control leaves is slightly greater than the value for A calculated here This is because the Pt_n (con) value (equation 3) is the net amount of chlorophyll after a proportion has broken down This proportion cannot be determined and so its omission

Table 3 Calculated values for chlorophyll degradation (*A*) and synthesis (*B*), rate constants and half-lives in greening leaves

Light treatment (hr)	Chlorophyll nmol/leaf/7 hr		Turnover rate constants pmol/nmol chlorophyll/hr		Chlorophyll half-life (hr)	
	Degradation (*A*)	Synthesis (*B*)	Degradation (*Ak*)	Synthesis (*Bk*)	*x*	*y*
2	3 6	8 9	84 1	127 2	6 6	8 2
3	6 1	9 3	105 3	100 6	6 7	6 9
6	10 5	14 4	101 8	99 5	6 9	7 0
12	7 9	11 2	44 5	50 6	14 6	15 6
24	6 8	11 3	26 7	38 0	21 4	26 0
48	8 7	11 4	19 1	22 5	33 3	36 3
60	5 6	7 2	10 8	13 2	57 7	64 1

Values for *A* and *B* were calculated from the data in Tables 1 and 2 using equations 2 and 3, rate constants (*Ak*) and (*Bk*) were calculated using equation 4 and half-life (half-time) values from equation 5 using either the mean of rate constants *Ak* and *Bk* (*x*) or the lower value of *Ak* and *Bk* (*y*)

means that the values, calculated for *A* and *B* are a slight underestimate It should also be emphasized that the results described here are for leaves excised for the last 14 hr There is some evidence that detached leaves have a slower rate of chlorophyll destruction, at least when placed in the dark [18]

Virgin [8] reported a rapid photodestruction of chlorophyll particularly during the lag-phase in pigment production. Certainly, in the experiments reported here, the turnover of chlorophyll, during the first few hours of greening, is a relatively rapid process The turnover of chlorophyll in *Chlorella* [13] and the diatom, *Skeletonoma* [12] was also found to be very rapid with values of 1 hr and 3–10 hr, respectively In higher plants, however, turnover times in the order of days, have been suggested [19–24] for relatively mature and fully greened leaves

It is possible that LA treatment may modify chlorophyll breakdown LA treatment was found to slow down protochlorophyllide breakdown in barley [16] and may or may not have other effects on primary metabolism [22, 23] Nonetheless the use of LA, as an inhibitor in chlorophyll synthesis, provides a convenient method for the estimation of chlorophyll turnover in greening leaves

EXPERIMENTAL

Wheat seeds (*Triticum vulgare*) were soaked in water for 16 hr and sown in moist vermiculite The seedlings were grown in the dark at 240° After 6 days the seedlings were transferred to the light (Fluorescent Atlas Super-White, 65/80 watt, with an illuminance at seedling level of 3500 lux) Laevulinic acid soln (0 1 M in 0 11 M phosphate buffer, pH 7 2) was prepared as required from the desiccated compound Chlorophyll was extracted in 80% aq Me_2CO containing a small quantity of Na_2CO_3 and estimated using the equations of Arnon [24] Rate constant and half-time calculations were based on definitions and equations presented by Reiner [25] and Atkins [26] *Ak* and *Bk* values were calculated from equation 4

$$K = [A(\text{or } B)]/t \times 1/[(Pt_7 + Pt_0)/2] \quad (4)$$

Half-life (half-time) values were calculated from

$$Kt_{1/2} = \ln 2 \quad (5)$$

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