Plant Physiol. (1975) 56, 497-501

# Control of δ-Aminolevulinic Acid and Chlorophyll Accumulation in Greening Maize Leaves upon Light-Dark Transitions[1]

Received for publication March 21, 1975 and in revised form June 10, 1975

ROBERT FLUHR, EITAN HAREL, SHIMON KLEIN, AND ERNA MELLER
*Department of Botany, The Hebrew University, Jerusalem, Israel*

## ABSTRACT

**The accumulation of δ-aminolevulinic acid (ALA) was studied in greening maize (*Zea mays*) leaves which were transferred to darkness and reilluminated after various periods of time. The system synthesizing ALA decays in the dark with a half-life of about 80 minutes. The onset of enzyme decay after transfer to darkness shows a 40-minute lag. The accumulation of ALA in the presence of levulinic acid in leaves transferred to darkness corresponds to that expected from the estimated half-life of the enzyme synthesizing ALA. On the other hand, the accumulation of protochlorophyll upon transfer to darkness in the absence of levulinic acid stops much earlier. It is suggested that a control point exists in the pathway between ALA and protochlorophyll, preventing utilization of the accumulated ALA upon transfer of greening leaves to darkness. This is supported by the observed effects of low intensities of monochromatic light (648 nm) on ALA and chlorophyll accumulation.**

The enzyme responsible for the synthesis of ALA[2] has been ascribed a central role in the regulation of Chl synthesis in higher plants (6, 7, 12, 13). It has been suggested that all of the enzymes situated in the pathway between ALA and Pchl are present in nonlimiting amounts in etiolated leaves (6, 7, 12, 13, 23). Chl accumulation would then be dependent on the rate of ALA production. The induction of ALA and Chl synthesis as well as their continued accumulation are light-dependent and require protein synthesis (2, 7, 10, 14, 19, 20, 25). Two lines of hypotheses have been put forward to explain the role of light in controlling ALA synthesis. (*a*) The enzyme responsible for the synthesis of ALA has a short half-life and its resynthesis is light-dependent (7, 10, 19). Estimates for the half-life of the enzyme, based on Chl determinations, range from several minutes (25) to 1.5 hr (19). (*b*) The activity of the enzyme is controlled through feedback inhibition by Pchl (15, 16, 22) or some other intermediate (5, 8), the level of which is light-dependent. In these cases, the involvement of protein synthesis is indirect (5, 15, 16).

It has been shown that, under certain conditions, the accumulation of ALA measured in the presence of LA is closely related to Chl synthesis (1, 3, 17, 20, 21). In these cases, it is possible to study problems related to the control of Chl synthesis by following the accumulation of its early precursor, ALA, without having to depend on measurements of the end product, Chl, alone.

We have studied the kinetics of ALA and Chl accumulation in greening maize leaves during light-dark transitions in an attempt to understand the mechanism by which light controls the synthesis of ALA and Chl. Maize leaves seem to be a good choice for this purpose for a number of reasons. (*a*) The accumulation of ALA and Chl is linear for a relatively long period of time in detached etiolated leaves subjected to illumination and the lag phase in their accumulation is relatively short (9, 14). (*b*) A 1:1 ratio exists between the amount of ALA accumulated and that of Chl "missing," when Chl synthesis is inhibited by LA, over a wide range of experimental conditions (LA concentration, seedling age, light intensities, etc.) (9, 17). (*c*) ALA production is apparently not limited by the supply of precursors during the early stages of greening (17). (*d*) LA has apparently no direct effects other than the inhibition of ALA utilization for porphyrin synthesis (17).

## MATERIALS AND METHODS

ALA and levulinic acid (grade I) were purchased from Sigma Chemical Co., acetylacetone from B.D.H. Chemicals Ltd., Poole, Dorset, England and 4-dimethylamino-benzaldehyde from E. Merck A.G. LA was purified according to Beale (1). Seeds were obtained as previously reported (14). Growing conditions, application of LA and ALA, incubation conditions, and extraction and determination of ALA and pigments were carried out as previously described (14, 17). Duplicate or triplicate treatments were carried out in each experiment. Manipulation of leaves in the "dark" was performed under a green safe-light, inactive in transforming Pchl. At the end of the treatment period, leaves were dropped into liquid nitrogen before grinding. Leaf samples for Pchl determination were ground under a green safelight. Monochromatic light (648 nm) was provided through a Balzers Filtraflex B-20 filter.

## RESULTS

**Accumulation of ALA and Pchl following Transfer of Greening Leaves to Darkness.** Maize leaves were illuminated for 4 hr with 80 ft-c of white fluorescent light in the presence of 50 mM LA, returned to darkness, and the accumulation of ALA and Pchl was then followed. During the first 15 to 30 min, the rate of Pchl accumulation in untreated leaves was similar to that of Chl in continuous light (140 nmoles ALA/g fresh weight·hr) but leveled off rapidly thereafter. In LA-treated leaves, Pchl accumulated at a slower rate (about 50% of the rate in untreated leaves, during the first 30 min in darkness) as expected due to the inhibitory effect of LA (Fig. 1). In both cases, the amount of Pchl

[1] This work was supported by a grant from the Israel Commission for Basic Research.

[2] Abbreviations: ALA: δ-aminolevulinic acid; LA: levulinic acid; Chl: chlorophyll(ide); Pchl: protochlorophyll(ide); DOVA: γ,δ-dioxovaleric acid.

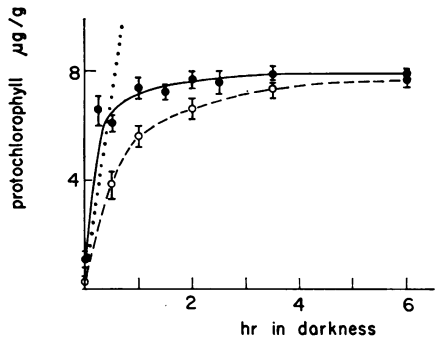


FIG. 1. Accumulation of PChl after the transfer of greening leaves to darkness in the absence and presence of LA. Ten-day-old etiolated maize leaves were incubated in the dark with (○) or without (●) 50 mM LA for 2 hr, illuminated for 4 hr with 80 ft-c of white fluorescent light and returned to darkness. Chl in continuous light, no LA (····).

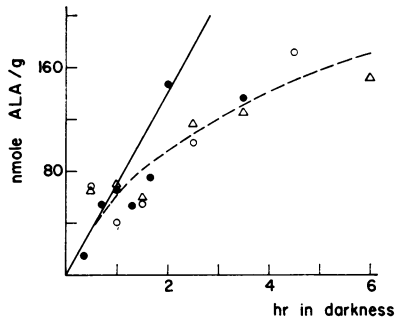


FIG. 2. Accumulation of ALA after transfer of greening maize leaves to darkness. Treatment of leaves as in Fig. 1, 50 mM LA. Results from three independent experiments (○, ●, △), each point is the mean value of two or three leaf samples. The line for ALA accumulation following the transfer to darkness (– – –) is a result of a nonlinear least squares curve-fitting procedure according to a function predicting ALA accumulation under these conditions. This was based on the assumption that the amount of ALA found in the leaves equals the difference between the amount synthesized and that utilized for making Pchl. The rate of ALA formation following the transfer of greening leaves to darkness was assumed to be a function of the monomolecular decay of the system producing ALA (see equation 7 in the text). ALA accumulation in continuous light (×——×).

reached 7 to 8 μg/g fresh weight, and no significant increase was observed, after an additional 4 hr in the dark. This is the amount of Pchl usually found in 10-day-old etiolated maize leaves. ALA accumulated at a decreasing rate in LA-treated leaves after their return to darkness for at least 4 hr (Fig. 2). The rate of ALA accumulation during the first 30 to 50 min in darkness equaled that of leaves which remained in continuous light. It is evident from the data shown in Figures 1 and 2 that the amount of ALA found after return to darkness was larger than the difference between Pchl (expressed as ALA) in the controls and in LA-treated leaves.

The decreasing rate of ALA accumulation upon transfer of greening leaves to darkness could result either from the decay of the enzyme synthesizing ALA or from progressive inhibition of enzyme activity in darkness caused by the accumulation of an inhibitor. It may be feasible to differentiate between the two possibilities by following the accumulation of ALA after light-dark-light transitions.

**ALA Accumulation upon Light-Dark-Light Transitions.** LA-treated maize leaves were exposed to light for 3 hr, transferred to darkness, and reilluminated after various periods of time in the dark. If the cessation of ALA production in the dark were due to the inhibition of enzyme activity by the accumulating Pchl, a

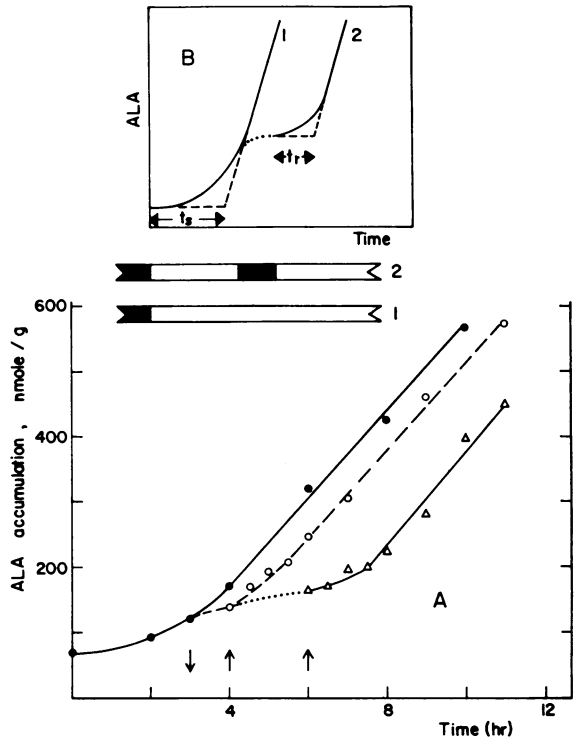


FIG. 3. ALA accumulation in maize leaves during light-dark-light transitions. A: Ten-day-old etiolated leaves were preincubated in the dark for 2 hr with 50 mM LA and then transferred to light (80 ft-c). While one set of leaf samples remained in continuous light (●), a second set was transferred to the dark for 1 hr (○), and a third for 3 hr (△) before being reilluminated. Arrows indicate transfer to darkness (↓) or light (↑). B: Schematic representation of the analysis of the results in 3A, showing illumination regimes and the time required to reach the steady state rate of ALA accumulation ($t_s$ and $t_r$).

rapid resumption would be expected upon reillumination, of the steady state rate (rate in continuous light) of ALA accumulation. In this case, the resumption would be independent of the length of the preceding dark period, since Pchl is rapidly transformed to Chl upon reillumination. If enzyme decay were involved, the time required for the resumption of full accumulation rate would depend on the length of the dark period, up to the point where all the enzyme has decayed.

Figure 3A shows the accumulation of ALA under such experimental conditions. A delay in the resumption of the steady state rate of ALA accumulation is evident.The time needed for this resumption was clearly dependent on the length of the preceding dark period (Fig. 3A). This indicates a lability of a component in the ALA synthesizing system. A quantitative analysis of these data requires some assumptions. (*a*) The lability in the dark can be expressed as a monomolecular decay of an enzyme, presumably the ALA synthesizing enzyme. (*b*) Resynthesis of the labile component is light-dependent.

The amount of enzyme remaining after a period of darkness can be expressed as:

$$E = E_{ss} \exp(-k_D t_D) \quad (1)$$

where $E_{ss}$ is the steady state level of the enzyme in continuous light, ($E$ is defined from 0 to 1 in relative units), $k_D$ is the decay constant of the enzyme in darkness and $t_D$ is time the leaves remained in darkness.

The amount of enzyme that has to be resynthesized upon reillumination ($E_r$) would be:

$$E_r = E_{ss}[1 - \exp(-k_D t_D)] \quad (2)$$

The time needed for resynthesis, when no enzyme is initially present ($t_s$), can be ascertained from the first exposure of etiolated leaves to light and approximated by a linear function of time, so that:

$$E_{ss} = k_s t_s \quad (3)$$

where $k_s$ is the rate constant of the synthesis of the enzyme. Assuming that the events controlling the initial lag in ALA synthesis are the same as those involved in the case of light-dark-light transitions:

$$E_r = k_s t_r \quad (4)$$

where $t_r$ is the time required to reach $E_{ss}$, when greening leaves are reilluminated after a short period in darkness.

Substituting equations 3 and 4 in equation 2, obtains

$$t_r = t_s[1 - \exp(-k_D t_D)] \quad (5)$$

Thus:

$$\ln\left|\frac{t_s - t_r}{t_s}\right| = -k_D t_D \quad (6)$$

In order to obtain $t_s$ and $t_r$ from the experimental results, the time interval is taken from the return of leaves to the light until the linear rate of ALA accumulation is resumed (Fig. 3B).

Figure 4 is a log plot of the values of $(t_s - t_r)/t_s$ obtained in four experiments of the kind shown in Figure 3A. Values for $t_s$ varied from 1.75 to 2.25 hr. The best fit values from these results gave $k_D = 0.53$ hr$^{-1}$ with a coefficient of correlation of 0.90. This value of $k_D$ is equivalent to a half-life of 78 min of the ALA-synthesizing system. An interesting point emerging from the above analysis is that there was a significant lag (about 40 min) between the times leaves were transferred to darkness and the apparent onset of enzyme decay. This can be seen from the intercept of the regression line with the time axis. Equation 5 therefore requires an empirical correction.

The accumulation of ALA during the first 30 to 50 min after the transfer of greening maize leaves to darkness (Fig. 2) is compatible with a delay in the onset of enzyme decay in darkness. The existence of such a delay is supported by the observation that ALA accumulation in maize leaves illuminated for 2 min every 4 or 28 min was not different from that of leaves which remained in continuous light (Fig. 5). Had enzyme decay followed immediately on transfer to darkness, intermittent light would have had a marked effect on ALA accumulation.

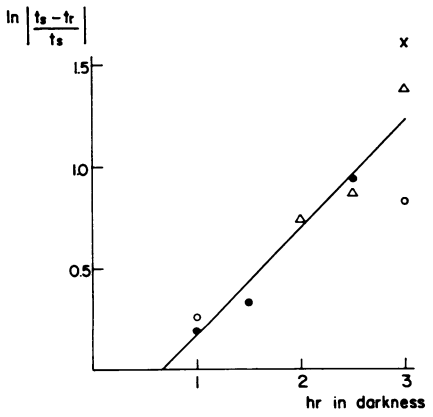


FIG. 4. Analysis of results on ALA accumulation during light-dark-light transitions. Results from 4 experiments like the one shown in Figure 3. $t_s$: Time required to reach the steady state rate of ALA accumulation when no enzyme is initially present (first exposure to light); $t_r$: time required after various periods of time in the dark. The slope of the regression line (coefficient of correlation = 0.90) gives an estimate for the decay constant of the enzyme(s) synthesizing ALA.

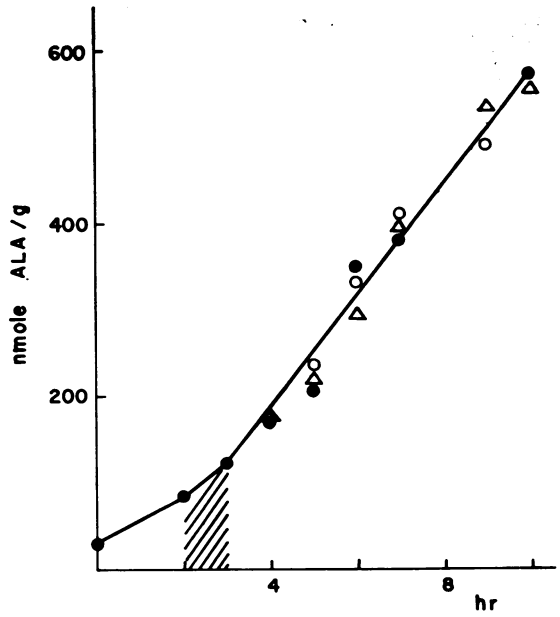


FIG. 5. Accumulation of ALA during intermittent illumination of maize leaves. Ten-day-old etiolated leaves were preincubated for 2 hr in the dark with 50 mM LA and transferred to 80 ft-c of white fluorescent light for 2 hr, returned to darkness for 1 hr (shaded area), and then re-illuminated with 80 ft-c continuously (●), for 2 min every 4 min (○), or every 28 min (△).

**Control by Feedback Inhibition or Enzyme Decay.** We can now predict the total amount of ALA (ALA and Pchl) expected to accumulate in leaves after transfer from light to darkness if only enzyme decay is involved. Since the rate of ALA production is proportional to the concentration of the enzyme, the integration of the empirically corrected equation 1 yields:

$$\text{ALA produced} = \frac{k_l E_{ss}}{k_D}[1 - \exp(-k_D[t_D - t_l])] \quad (7)$$

where $k_l E_{ss}$ represents rate of total ALA production in continuous light (estimated from the rate of Chl accumulation in leaves not treated with LA: 140 nmoles/g fresh weight·hr) (8) and $t_l$ − the lag of 40 min observed before enzyme decay begins. To this, the amount of ALA which the system will produce during the 40-min lag period should be added: ALA produced during lag $= k_l E_{ss} t_l = 140 \times 0.67 = 93$ nmoles/g fresh weight. Since the inhibitory effect of LA on Chl accumulation appears to be the same in light and darkness (Fig. 1) (17), the rate of ALA accumulation in continuous light in the presence of LA (70 nmoles/g fresh weight·hr) can be used to calculate from equation 7 the amount of ALA expected to accumulate in darkness, in the presence of 50 mM LA. Figure 6 shows the amounts of ALA and Pchl expected to accumulate and experimentally observed (data of Figs. 1 and 2) after transfer of maize leaves from light to darkness. It can be seen that there are discrepancies between the amounts of total ALA found (curves B and E, Fig. 6) and the amount expected (curve A) in both LA-treated and non-treated leaves. The discrepancy is most pronounced in non-treated leaves where the amount of ALA accumulated as Pchl after 4 hr in the dark (curve E) is about 70 nmoles/g while the expected amount is 311 nmoles/g fresh weight. The amount of ALA which accumulated in the presence of LA (curve D) closely approached the amount predicted (curve C, Fig. 6). The difference between the total amount of ALA expected to be made and that actually observed in the tissue thus seems to be

related to the early cessation of Pchl accumulation which reaches the same level in both treated and untreated leaves. This early cessation of Pchl accumulation upon transfer to darkness may not be due solely to the decay of the enzyme responsible for ALA synthesis. It could also result from inhibition by Pchl, or a compound related to it, of a step between ALA and Pchl. The effect of low intensities of monochromatic light, effective in the conversion of Pchl to Chl (648 nm), on ALA and Chl accumulation supported this possibility (Fig. 7). Maize leaves were preincubated for 2 hr in the dark with or without LA, illuminated for 2 hr with 80 ft-c of white fluorescent light to induce ALA production, and returned to darkness. After 30 min in the dark, during which Pchl accumulated, the leaves were illuminated for 4 hr with various intensities at 648 nm. The amounts of ALA and Chl which accumulated in monochromatic light were estimated after subtracting the amounts found in leaves which remained in the dark during the 4-hr period. It can be seen from Figure 7 that ALA accumulation was saturated at lower light intensities than Chl accumulation in either LA or $H_2O$. The ratio of total ALA (ALA + Chl), produced in the presence of LA to total ALA formed in the $H_2O$ controls as Chl, decreased from 5 in the lowest intensity used to 1, when Chl formation approached saturation (Fig. 8). This might indicate the existence of a control point between ALA and Chl since, although ALA could accumulate at low light intensities, its utilization for Chl was inhibited unless the intensity was increased. Utilization of the ALA formed could thus be prevented by a certain level of Pchl and allowed to proceed after part of it is photoconverted.

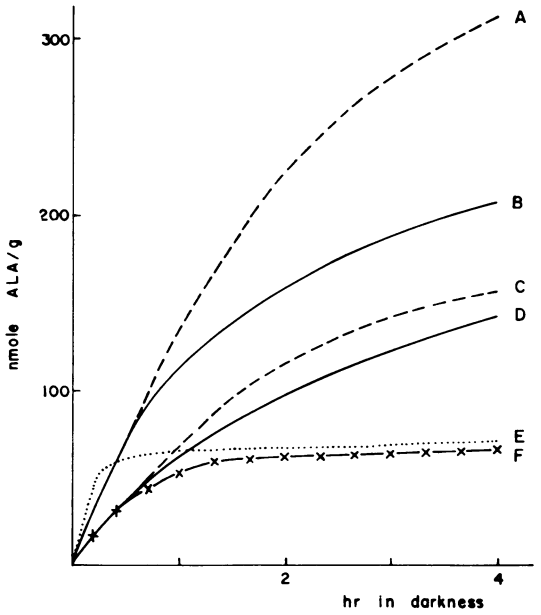


FIG. 6. Accumulation of Pchl and ALA after transfer of greening maize leaves to darkness—comparison of "expected" and experimentally observed data. A: Total ALA (ALA and Pchl expressed as ALA) expected to accumulate according to the estimated half-life of the enzyme synthesizing ALA; B: observed accumulation of total ALA (ALA + Pchl) after transfer to darkness; C: ALA expected to accumulate according to the estimated half-life of the enzyme synthesizing ALA, in the presence of 50 mM LA; D: observed accumulation of ALA after transfer to darkness, in the presence of 50 mM LA; E: Pchl in $H_2O$ controls; F: Pchl in leaves treated with 50 mM LA. B, D, E, and F were taken from the experimental results shown in Figs. 1 and 2.

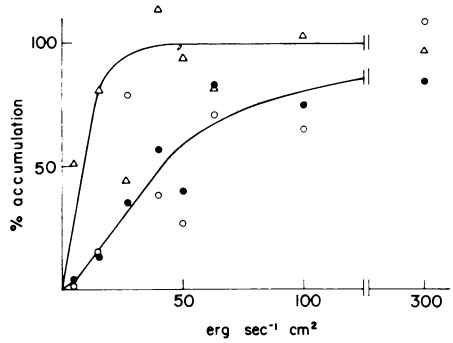


FIG. 7. Accumulation of ALA and Chl in monochromatic light (648 nm) of various intensities. Ten-day-old etiolated maize leaves were preincubated for 2 hr in the dark with or without 50 mM LA, illuminated for 2 hr with 80 ft-c of white fluorescent light, to fully induce ALA formation, and returned to darkness for 30 min. The leaves were then illuminated for 4 hr with various intensities at 648 nm. The amount of ALA and Chl which accumulated in monochromatic light were estimated after subtracting the amounts found in leaves which remained in the dark during the 4-hr period. Results are from six independent experiments. In each experiment, pairs of leaf samples were exposed to three different light intensities. Each point is the mean of four leaf samples. Chl and ALA are expressed as percentage of the amount accumulated under 670 erg/cm² sec in the particular experiment. ALA in LA-treated leaves (△——△); Chl in LA-treated leaves (○); and in $H_2O$ controls (●).

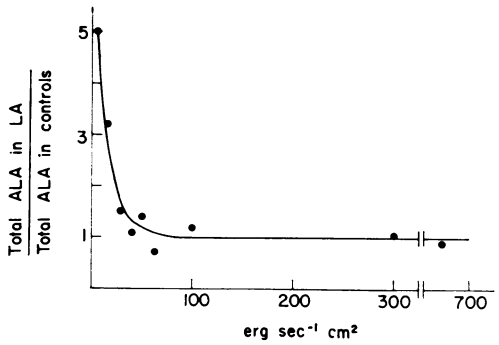


FIG. 8. Ratio between total ALA accumulated in the presence of LA and in $H_2O$ controls at various intensities of monochromatic light (648 nm). Calculated from the results of the experiments described in Fig. 7. Total ALA = nmoles ALA + 8 nmoles Chl and Pchl.

## DISCUSSION

Analysis of the kinetics of ALA accumulation upon light-dark-light transitions showed that the enzyme(s) responsible for the synthesis of ALA has indeed a relatively short half-life as has been suggested by several workers (2, 3, 7, 10, 19). Therefore, the dependence of ALA accumulation on continued protein synthesis (17) could be explained at least in part, as the requirement for synthesis of the enzyme catalyzing ALA formation. The level of the enzyme is apparently controlled by a compound which accumulates within 40 min of the transfer of greening leaves to darkness and arrests enzyme synthesis. The inhibition of enzyme synthesis is rapidly relieved upon reillumination. This renders Pchl, or a compound related to it, a likely candidate for being the inhibitor (repressor?), although the involvement of phytochrome could not be excluded. Alternatively, a compound required for the synthesis might gradually disappear following the transfer of greening leaves to darkness. The fact that intermittent illumination with up to 28-min dark periods did not depress ALA accumulation (Fig. 5) suggests that the system synthesizing the ALA-producing enzyme is relatively stable in the dark and resumes activity upon reillumination and removal of the inhibitor.

The half-life of the ALA-producing enzyme is not sufficiently short to account for the rapid cessation of Pchl accumulation

upon the transfer of greening leaves to darkness. This leaves room for feedback inhibition by Pchl, or some other compound which accumulates in the dark, on either ALA formation or a later step in the pathway to Chl. Feedback inhibition of the ALA-synthesizing enzyme has been suggested by Schiff and Epstein (22) and is indicated by the results of Beney and Nigon (5) in *Euglena* and Duggan and Gassman (8) in bean leaves. A similar mechanism might operate in maize leaves, preventing further production of ALA when a certain level of Pchl is reached and before the system producing ALA has completely decayed. This will explain inability to detect ALA upon return to darkness in the absence of LA. The fact that in the presence of LA more ALA is produced than in its absence could then be due to lower levels of Pchl formed in the presence of LA (Fig. 1). In this case Pchl will not accumulate to effective inhibitory levels during the first few hours in darkness. The kinetics of ALA and Pchl accumulation upon transfer of greening leaves to darkness in the presence and absence of LA tend to disfavor feedback inhibition on ALA formation in maize leaves (Fig. 6). Not only does ALA continue to accumulate after Pchl levels off, but the rate of accumulation closely follows that expected from the estimated half-life of the system (Fig. 6). The discrepancy between the amount of Pchl expected and that observed must therefore be due to a control point located between ALA and Pchl. It seems likely that an enzyme(s) situated in the pathway between ALA and Pchl is inhibited by a compound which accumulates in the dark and is "destroyed" by reillumination. This prevents the utilization of a considerable part of the ALA accumulated by the decaying system for Pchl production. The existence of such a mechanism operating between ALA and Pchl is also suggested by the differential effect of low intensities of monochromatic light of 648 nm on Chl and ALA accumulation (Fig. 7). If this is the case, accumulation of considerable amounts of ALA and/or porphyrins after transfer of greening leaves to darkness in the absence of LA would be expected. However, we were unable to detect either ALA or porphyrins under such conditions. Porphyrins, mainly protoporphyrin IX and Mg protoporphyrin, did accumulate when exogenous ALA was supplied at relatively high concentrations (10–40 mM) to maize leaves in the dark (Harel and Klein, unpublished results). Similar observations were reported earlier by Granick (12).

A possible explanation for the inability to detect ALA after transfer of greening leaves to darkness could be that in non-treated leaves the ALA which accumulates in the dark is rapidly diverted to another metabolic pathway. An alternate route of ALA metabolism was demonstrated by Shemin *et al.* (24) in avian erythrocytes. Troxler and Brown (26) suggested recently the existence of a major alternate route(s) of ALA metabolism in red and blue-green algae. The recent report by Beale and Castelfranco (4) and work in our laboratory (18) suggest that ALA might be synthesized in higher plants from $\alpha$-ketoglutarate or glutamate, via DOVA. Since the transamination of DOVA to ALA is a reversible process (11), ALA which has been accumulated in the dark might be converted back to DOVA when its utilization for Chl synthesis is inhibited. The fact that ALA does accumulate upon transfer to darkness in the presence of LA could then be due to inhibition by ALA of the enzyme which diverts ALA to the alternate route, or back to DOVA.

*Acknowledgments*—We wish to thank Prof. Dan Cohen for helpful discussions and Mrs. E. Katz and Mrs. E. Gan-Zvi for technical assistance.

## LITERATURE CITED


1. Beale, S. I. 1970. The biosynthesis of δ-aminolevulinic acid in *Chlorella*. Plant Physiol. 45: 504–506.
2. Beale, S. I. 1971. Studies on the biosynthesis and metabolism of δ-aminolevulinic acid in *Chlorella*. Plant Physiol. 48: 316–319.
3. Beale, S. I. and P. A. Castelfranco. 1974. The biosynthesis of δ-aminolevulinic acid in higher plants. I. Accumulation of δ-aminolevulinic acid in greening plant tissues. Plant Physiol. 53: 291–296.
4. Beale, S. I. and P. A. Castelfranco. 1974. The biosynthesis of δ-aminolevulinic acid in higher plants. II. Formation of $^{14}$C-δ-aminolevulinic acid from labeled precursors in greening plant tissues. Plant Physiol. 53: 297–303.
5. Beney, G. and V. Nigon. 1974. Action of cycloheximide on δ-aminolevulinic acid and chlorophyll production in *Euglena gracilis*. *In:* M. Avron, ed., Proceedings of the Third International Congress on Photosynthesis, Rehovot. Elsevier, Amsterdam. pp. 1801–1808.
6. Bogorad, L. 1966. The biosynthesis of chlorophylls. *In:* L. P. Vernon and G. R. Seely, eds., The Chlorophylls. Academic Press, New York. pp. 481–510.
7. Bogorad, L. 1968. Control mechanisms in plastid development. *In:* M. Locke, ed., Control Mechanisms in Developmental Processes. Academic Press, New York. pp. 1–31.
8. Duggan, J. and M. Gassman. 1974. Induction of porphyrin synthesis in etiolated bean leaves by chelators of iron. Plant Physiol. 53: 206–215.
9. Fluhr, R., E. Harel, S. Klein, and E. Neéman. 1974. The control of δ-aminolevulinic acid synthesis in greening maize leaves. *In:* M. Avron, ed., Proceedings of the Third International Congress on Photosynthesis, Rehovot. Elsevier-Amsterdam. pp. 2097–2103.
10. Gassman, M. and L. Bogorad. 1967. Control of chlorophyll production in rapidly greening bean leaves. Plant Physiol. 42: 774–780.
11. Gassman, M., J. Pluscec, and L. Bogorad. 1968. δ-Aminolevulinic acid transaminase in *Chlorella vulgaris*. Plant Physiol. 43: 1411–1414.
12. Granick, S. 1963. The pigments of the biosynthetic chain of chlorophyll and their interaction with light. *In*: N. M. Sissakian, ed., Fifth International Congress of Biochemistry, Moscow, Vol. 6. Pergamon Press, Oxford. pp. 176–185.
13. Granick, S. and S. Sassa. 1971. δ-Aminolevulinic acid synthetase and the control of heme and chlorophyll synthesis. *In:* H. J. Vogel, ed., Metabolic Regulation, Vol. V. Academic Press, New York. pp. 77–141.
14. Harel, E. and S. Klein. 1972. Light dependent formation of δ-aminolevulinic acid in etiolated leaves of higher plants. Biochem. Biophys. Res. Commun. 49: 364–370.
15. Kirk, J. T. O. 1968. Studies on the dependence of chlorophyll synthesis on protein synthesis in *Euglena gracilis*, together with a monogram for the determination of chlorophyll concentration. Planta 78: 200–207.
16. Kirk, J. T. O. and R. L. Allen. 1965. Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. Biochem. Biophys. Res. Commun. 21: 523–530.
17. Klein, S., E. Harel, E. Neéman, R. Katz, and E. Meller. 1975. Accumulation of δ-aminolevulinic acid and its relation to chlorophyll synthesis and development of plastid structure in greening leaves. Plant Physiol. 56: 486–496.
18. Meller, E., S. Belkin, and E. Harel. 1975. The biosynthesis of δ-aminolevulinic acid in greening maize leaves. Phytochemistry. In press.
19. Nadler, K. and S. Granick. 1970. Controls on chlorophyll synthesis in barley. Plant Physiol. 46: 240–246.
20. Oh-hama, T. and H. Senger. 1975. The development of structure and function in the chloroplasts of greening mutants of *Scenedesmus*. III. Biosynthesis of δ-aminolevulinic acid. Plant Cell Physiol. In press.
21. Richard, F. and V. Nigon. 1973. La synthèse de l'acide δ-aminolèvulinique et de la chlorophylle lors de l'éclairement d'*Euglena gracilis* étiolées. Biochim. Biophys. Acta 313: 130–149.
22. Schiff, J. A. and H. T. Epstein. 1966. The replicative aspect of chloroplast continuity in *Euglena*. *In:* T. W. Goodwin, ed., Biochemistry of Chloroplasts, Vol. I. Academic Press, London. pp. 341–353.
23. Schneider, H. A. W. 1973. Limitierende Proteine in der Chlorophyllbiosynthese. Experimente und Modellvorstellungen. Ber. Deutsch. Bot. Ges. 86: 431–436.
24. Shemin, D., C. S. Russel, and T. Abramsky. 1955. The succinate-glycine cycle. I. The mechanism of pyrrole synthesis. J. Biol. Chem. 215: 613–626.
25. Süzer, S. and K. Sauer. 1971. The sites of photoconversion of protochlorophyllide to chlorophyll in barley seedlings. Plant Physiol. 48: 60–63.
26. Troxler, R. F. and A. S. Brown. 1975. Metabolism of δ-aminolevulinic acid in red and blue-green algae. Plant Physiol. 55: 463–467.