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# Correlation between 5-Aminolaevulinate Accumulation and Protochlorophyll Photoconversion

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**Abstract.** A 1-min light pulse delivered to mustard seedlings (*Sinapis alba* L.) 60 h after sowing initiates the release of cotyledonary 5-aminolaevulinate (ALA) accumulation which continues for at least 2 h in the dark. Phytochrome ($P_{fr}$) increases the rate of ALA accumulation after a 24-h red light pretreatment but is not the trigger for this release. It is shown that the rate of ALA accumulation varies with the wavelength and fluence rate of the 1-min light pulse and can be predicted from the degree of protochlorophyll(ide) photoconversion. There is a linear correlation between the rate of ALA accumulation and the degree of protochlorophyll(ide) (PChl)→chlorophyll(ide) a (Chl a) photoconversion in etiolated seedlings. In seedlings pretreated with red light this correlation is non-linear and the rate increases more rapidly with increasing degrees of PChl→Chl a photoconversion. It is suggested that there may exist an interaction between $P_{fr}$ and PChl→Chl a photoconversion in controlling ALA accumulation.

**Key words:** 5-Aminolaevulinate – Chlorophyll(ide) – Phytochrome – Protochlorophyll(ide) – *Sinapis*.

## Introduction

In higher plants the capacity of the 5-aminolaevulinate (ALA)-forming system, which is considered to be a rate-limiting step in chlorophyll formation (Bogorad 1976), is controlled by phytochrome ($P_{fr}$). This is indicated by results obtained with seedlings of mustard (Masoner and Kasemir 1975), maize (Klein et al. 1977) and barley (Miller et al. 1979). Light also determines the onset and termination of ALA accumulation: When ALA accumulation is measured in the presence of laevulinate (LA) it is found to commence shortly after the onset of illumination. Termination of ALA accumulation follows after the return to darkness. Little is understood about this "on-off" control (see Klein et al. 1975; Fluhr et al. 1975).

In this context, protochlorophyll(ide) (PChl) is sometimes suggested to be the photoreceptor for circumstantial reasons. Its photoconversion indicates conditions favoring photosynthesis and therefore a demand for Chl-destined ALA production (Schiff and Epstein 1966). More directly, Klein et al. (1977) have shown that maize seedlings irradiated with a short red light pulse of various energy fluence rates accumulate ALA during a subsequent 4-h dark period to a similar extent to which PChl is photoconverted to Chl. However, the fluence rate dependency of PChl photoconversion was reported to be different from that of ALA formation. Hence, up till now it is questionable whether PChl is the photoreceptor involved in the release of ALA accumulation.

In the present paper we report results which demonstrate a close correlation between PChl→Chl a photoconversion and the release of ALA accumulation in mustard cotyledons. We varied the wavelength and fluence rate of a 1-min light pulse and compared the effect of such variation on PChl→Chl a photoconversion and the subsequent rate of ALA accumulation in the dark. Results were obtained which are in accordance with the idea that PChl→Chl a photoconversion is the signal for the release of ALA accumulation.

## Materials and Methods

*Standard Techniques and Light Sources.* The techniques for photomorphogenetic research with mustard seedlings were used (Mohr 1966). The seeds (*Sinapis alba* L.) were purchased in 1975 from the Asgrow Company, (Hamburg FRG). Seedlings were either grown in the dark at 25° C or for the first 24 h in standard red light (emission maximum at 656 nm, half bandwidth 18 nm, fluence

*Abbreviations:* ALA = 5-aminolaevulinate; Chl = chlorophyll(ide); PChl = protochlorophyll(ide); cp = cotyledon pair; LA = laevulinate

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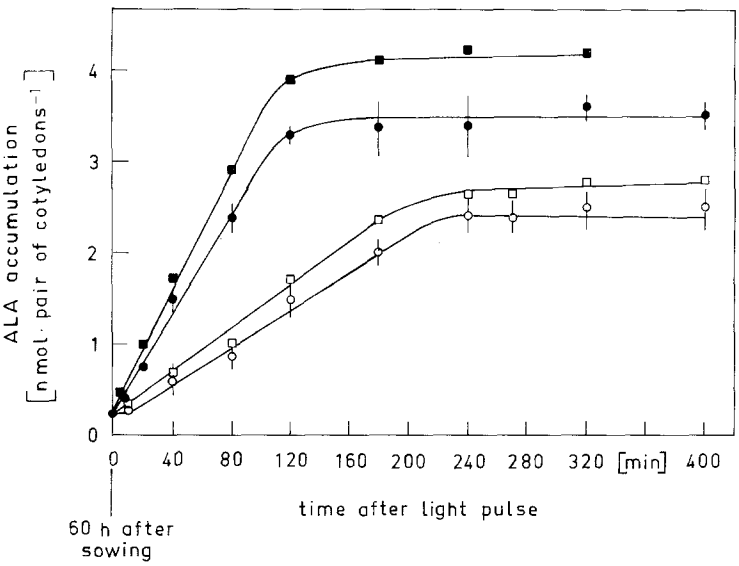


**Fig. 1.** ALA accumulation in the presence of laevulinate at 60 h after sowing. The seedlings were irradiated with 1 min white light (50 $Wm^{-2}$). ○● ALA accumulation alone. □■ ALA accumulation taking into account ALA incorporated into PChl ($ALA + ALA_{PChl}$) Open symbols, dark grown seedlings; closed symbols, seedlings pretreated with 24 h red light followed by 36 h dark

rate 0.68 $Wm^{-2}$) followed by 36 h of darkness. Light pulse treatment was given at 60 h using a modified Leitz Prado. The following filters were used to adapt the Prado for red, blue, and green light: red light up to 3 $Wm^{-2}$, DIL interference filter ($\lambda_{max}$ 658 nm, half bandwidth 10 nm); red light above 3 $Wm^{-2}$, AL interference filter ($\lambda_{max}$ 655 nm, half bandwidth 24 nm); blue light up to 1 $Wm^{-2}$, DIL interference filter ($\lambda_{max}$ 442 nm, half bandwidth 7 nm); blue light above 1 $Wm^{-2}$, AL interference filter ($\lambda_{max}$ 437 nm, half bandwidth 18 nm); green light, DIL interference filter ($\lambda_{max}$ 544 nm, half bandwidth 12 nm); 756 nm light, 7 $Wm^{-2}$, AL interference filter ($\lambda_{max}$ 756 nm, half bandwidth 11 nm). Interference filters were obtained from Schott (Mainz, FRG). The white light pulse was obtained from a tungsten bulb combined with heat-absorbing and neutral-glass filters. Continuous standard white light was obtained from fluorescent white light tubes (Osram, alternating 40 W/15 and 40 W/25) at an illumination of 3,500 lx.

*Extraction and Determination of ALA.* These were performed in principle according to Masoner and Kasemir (1975) using the method developed by Beale (1970). Seedlings were treated with a concentration of LA of 85 mM. Using this optimum concentration (Masoner and Kasemir 1975), inhibition is still incomplete due to competition between LA and ALA for 5-aminolaevulinate dehydratase. It can be seen from Fig. 1 that the rate of ALA accumulation in the presence of LA corresponds to approximately an 80% inhibition of ALA metabolism. The actual rate of ALA formation is thus about 20% higher than the measured ALA accumulation rate. This relationship between "measured" and "real" ALA accumulation was found to hold true for etiolated and red light pretreated seedlings (Fig. 1). The rate of ALA accumulation was estimated by treating the seedlings with LA solution 0.5 h before the onset of irradiation at 60 h. Accumulation of ALA was measured at several different time periods following the onset of light treatment, and the rate was calculated by linear regression analysis. The 95% confidence limits for the regression line between 20 and 100 min after the light pulse were calculated and are shown as bars in the figures. ALA accumulation was found to be linear during the periods used for rate measurements.

*Extraction and Determination of Chl and PChl.* The pigments were extracted with 80% acetone. Determination of Chl was performed after Ziegler and Egle (1965). Percentage PChl photoconversion during the 1-min light pulse was determined by comparing the Chl a present after the pulse with that present after a white light pulse which saturates for photoconversion (50 $Wm^{-2}$). An allowance was made for the trace of Chl a found in both etiolated and red light pretreated cotyledons immediately before the 1-min light pulse. The determination of regenerated PChl has been described elsewhere (Jabben et al. 1974). For calculation of the amount of ALA incorporated into PChl (Fig. 1), the amount of regenerated PChl was multiplied by 8 ($ALA_{PChl}$). The use of the biological unit "cotyledon pair" as a suitable system of reference has been justified repeatedly (see Kasemir et al. 1976).

## Results and Discussion

*Accumulation of ALA in Continuous White Light and in the Dark Following a 1-min White Light Pulse.* Masoner and Kasemir (1975) have shown that the rate of ALA accumulation in continuous white light can be increased by pretreating mustard seedlings with long-term far-red light which is considered to operate through phytochrome ($P_{fr}$). A similar result is obtained when mustard seedlings are pretreated with 24 h red light (Fig. 2) given during a period of time in which the ALA forming system is hardly developed (Kasemir et al. 1976). The preirradiated seedlings exhibit a constant high rate of ALA accumulation (2.8 nmol $cp^{-1}$ $h^{-1}$) from the onset of continuous white light. In contrast, the etiolated seedlings show initially a period with a lower rate of about 0.6 nmol $cp^{-1}$ $h^{-1}$, corresponding to the so-called "lag phase" in Chl a accumulation. The rate increases sharply after 2 h.

When the etiolated seedlings are treated with 1 min white light at 60 h after sowing, kept in the dark for 2 h, and then treated with continuous white light, the rate of ALA accumulation is nearly the same as in seedlings treated with continuous white light from 60 h after sowing. This effect of a short period of preirradiation is also attributed to the action of $P_{fr}$ (Masoner and Kasemir 1975).

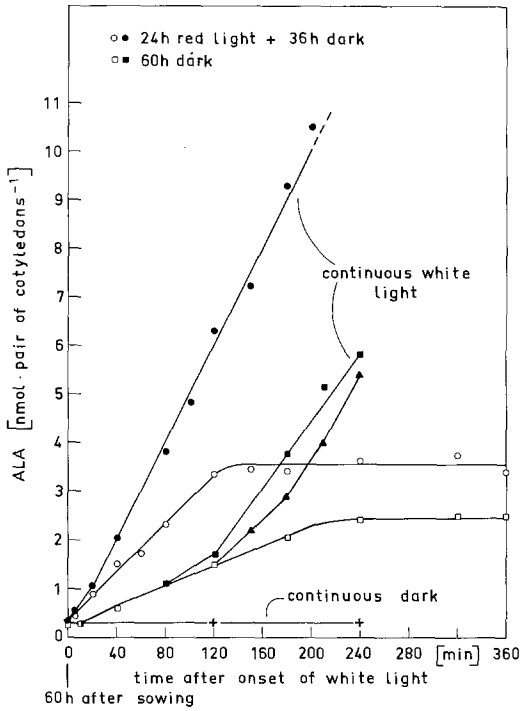


**Fig. 2.** ALA accumulation under continuous standard white light (closed symbols) and following 1 min white light (50 $Wm^{-2}$) saturating for PChl photoconversion (open symbols). ○● seedlings pretreated with 24 h red light plus 36 h dark, □■ dark grown seedlings. Onset of white light 60 h after sowing, ▲ 1 min white light (50 $Wm^{-2}$) at 60 h after sowing followed by 2 h dark and then by continuous standard white light. +, dark control. (Values are the same for etiolated and red light pretreated seedings)

Following the 1-min light pulse in the dark, the preirradiated seedlings accumulate ALA for about 2 h and the etiolated seedlings for about 4 h (Fig. 2). Thereafter a further ALA build-up ceases. These findings correspond in principle to previous reports describing the time course of ALA accumulation in the dark (Fluhr et al. 1975; Gough 1978; Miller et al. 1979). The ALA accumulation rate in continuous white light is higher than in the dark (Fig. 2). This indicates that some limitations of the ALA forming system are present when the seedlings are treated only with the 1-min light pulse. In red light pretreated seedlings these limitations become evident very rapidly (at least 20 min after the onset of irradiation), while in etiolated seedlings the differences in the rate are not obvious until after 2 h. We suggest that the provision of photosynthetic products could account for the higher ALA accumulation rate in continuous light in accordance with the proposed models for an ALA-producing system involving the concerted action of a kinase, dehydrogenase, and transaminase (Kannangara and Gough 1978, Lohr and Friedmann 1976).

*Photoconversion of PChl→Chl a During 1 min Light.* In accordance with Koski et al. (1951) and Björn (1975), we consider PChl to be the photoreceptor for its own photoconversion. The degree of PChl→Chl a photoconversion effected by a short light pulse is fluence-rate and wavelength dependent and follows a saturation curve (Fig. 3). Red light is more effective than blue light which in turn is more effective than white light in promoting PChl→Chl a photoconversion. It should be mentioned that the in vitro absorption spectrum of PChl predicts blue light to be the most effective. Its reduced influence reported here is considered to be the result of preferential blue light absorption by carotenoids (Koski et al. 1951).

*The Initiation of ALA Formation by 1 min Light.* To test the correlation between the degree of PChl→Chl a photoconversion and the subsequent rate of ALA formation, etiolated as well as red light pretreated seedlings were treated with 1 min light 60 h after sowing. The light pulse was varied in wavelength and fluence rate. As seen in Fig. 4, ALA accumulation is initiated by different qualities of light. In addition, the rate of ALA accumulation varies depending on the applied fluence rate. Increasing fluence rates are accompanied by increasing rates of ALA accumula-

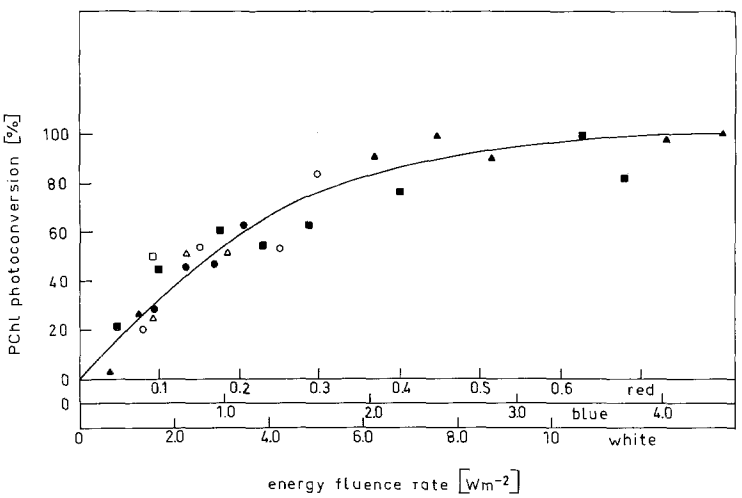


**Fig. 3.** Percentage PChl→Chl a photoconversion during 1 minute of red (○●), blue (□■) or white light (△▲) of different fluence rates. The curve is the same for etiolated (open symbols) and red light pretreated (closed symbols) seedlings

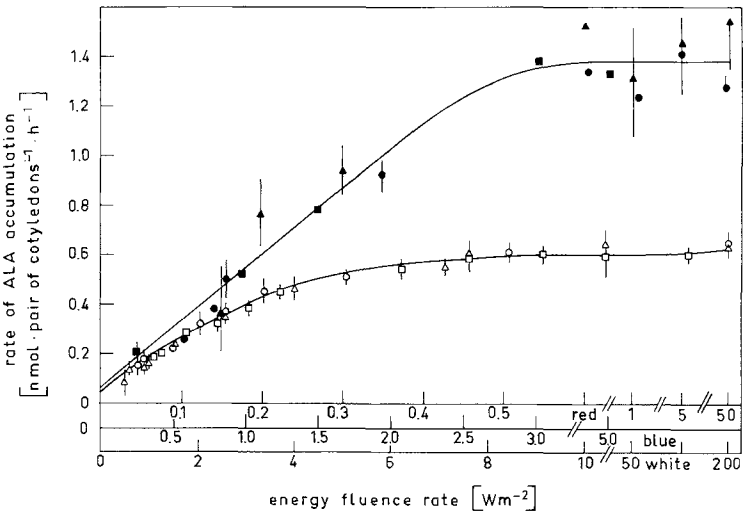


**Fig. 4.** Rates of ALA accumulation following 1 min of red (○●), blue (□■) or white light (△▲) of different fluence rates. Open symbols, etiolated seedlings; closed symbols, red light pretreated seedlings. The light pulse was given 60 h after sowing. Values for etiolated seedlings obtained from Belke (1979)

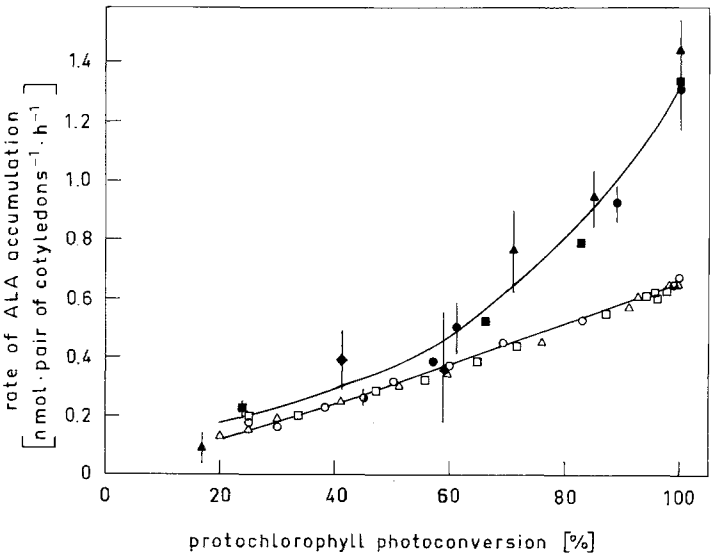


**Fig. 5.** ALA accumulation rates as a function of percentage PChl→Chl a photoconversion following 1 min light given at 60 h after sowing. Symbols as in Fig. 4. Data taken from Figs. 3 and 4. ◆, green light pulse, 1.0 $Wm^{-2}$

tion up to a limiting rate, beyond which further increases in fluence rate have no effect. This maximum rate is about 1.4 nmol $cp^{-1}\ h^{-1}$ in preirradiated cotyledons and about 0.6 nmol $cp^{-1}\ h^{-1}$ in etiolated material. It is independent of the wavelength of light used. It can be seen from Figs. 3 and 4 that the fluence rates achieving maximum PChl→Chl a photoconversion are about the same as those required for the attainment of a maximum ALA accumulation rate. The correlation between PChl→Chl a photoconversion and ALA accumulation is clearly seen when the ALA accumulation rate after a red, blue, or white light pulse is expressed in terms of PChl→Chl a photoconversion (Fig. 5).

As a further test of this correlation, we predicted the effect that a green light pulse should have on the ALA accumulation rate in red light pretreated seedlings by measuring its effect on PChl→Chl a photoconversion. The green light pulse converted 41% of the photoconvertible PChl. Figure 5 predicts a subsequent ALA accumulation rate of about 0.3 nmol

**Table 1.** Reversal experiments concerning the rate of ALA accumulation. Two programs were used: Program 1, etiolated seedlings were irradiated at 60 h after sowing with either 1 min red light (4.7 $Wm^{-2}$) and/or 3 min 756 nm far-red light (7.0 $Wm^{-2}$). Program 2, red light pretreated seedlings were irradiated with 4 white light flashes from a photographic electronic flash lamp distributed over 1 min, with or without simultaneously applying 756 nm far-red light during the 1 min light period

| | PChl→Chl a photoconversion | ALA [nmol cp $h^{-1}$] |
|---|---|---|
| Program 1 | | |
| 1 min red | 100 | 0.45±0.065 |
| 3 min 756 nm light | 0 | 0.00 |
| 1 min red+3 min 756 nm light | 100 | 0.42±0.092 |
| dark control | 0 | 0.00 |
| Program 2 | | |
| 4×white flash | 100 | 1.45±0.31 |
| 4×white flash with 756 nm background | 100 | 1.20±0.38 |
| 1 min 756 nm | 0 | 0.00 |

$cp^{-1}\ h^{-1}$. We measured the rate and found it to be $0.38 \pm 0.1$ nmol $cp^{-1}\ h^{-1}$. Thus, under all conditions tested, there appears to be a close relationship between PChl→Chl a photoconversion and the rate of ALA accumulation induced by the light pulse.

*Exclusion of a Possible Role for Phytochrome in the Initiation of ALA Accumulation.* To assess the extent to which $P_{fr}$ is involved in the initiation of ALA accumulation, we attempted to reverse the effect of a red or white light pulse by far-red light. We were unable to demonstrate the classical criterion for the involvement of phytochrome, namely the far-red light reversal of the induced light effect (Table 1). Thus, phytochrome appears not to be primarily involved in the initiation of ALA accumulation.

## Conclusions

Our results with mustard seedlings indicate that two photoreactions cooperate in the control of ALA accumulation by light, namely, the formation of $P_{fr}$ and the PChl→Chl a photoconversion. The enhanced ALA accumulation following a 24-h red light pretreatment shown in Fig. 2 is an expression of the phytochrome stimulated capacity for ALA accumulation (Masoner and Kasemir 1975; Kasemir et al. 1976). On the other hand, we were unable to demonstrate the involvement of $P_{fr}$ as the initiating factor in ALA accumulation (Table 1). Rather, the close correlation between the degree of PChl→Chl a photoconversion and the subsequent rate of ALA accumulation (Fig. 5), taken together with the evidence that PChl is the photoreceptor for its own photoreduction (Koski et al. 1951), suggest that PChl or the newly formed Chl a could be the photoreceptor for the release of ALA formation after a transfer from darkness to light.

Our limited in vivo data only allow speculation about the mechanism of the control of ALA accumulation, much of this having been summarized by Bogorad (1976). Independent of the underlying molecular events, our findings indicate the following properties of the control system in mustard cotyledons: There is a linear correlation between the rate of ALA accumulation and the degree of PChl→Chl a photoconversion in etiolated seedlings. However, in seedlings pretreated with red light this correlation is nonlinear. As seen in Fig. 5, the red light pretreatment leads to increasingly higher rates of ALA accumulation beyond 60% PChl→Chl a photoconversion, indicating that $P_{fr}$ increases the sensitivity of the ALA forming system to a signal produced by the PChl→Chl a photoconversion.

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