PHOTOCONVERSION OF CHLOROPHYLLIDE 684 TO CHLOROPHYLLIDE 678

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1. Introduction

The terminal steps of chlorophyll biosynthesis in higher plants consist of the phototransformation of protochlorophyllide to chlorophyllide and subsequent esterification with phytol. Spectroscopic examination of dark-grown leaves following irradiation gives information regarding the identity and order of formation of intermediate chlorophyllide species, which are designated by their wavelength maxima in nm. Molecular or structural details of the species which occur are unknown.

Shibata [1] spectroscopically observed the initial rapid photoconversion of protochlorophyllide (P)† to chlorophyllide (C) and two slow sequential dark spectral shifts:

$$P650 \xrightarrow{h\nu} C684 \xrightarrow{dark} C673 \xrightarrow{dark} C677$$

Gassman et al. [2], Sironval et al. [3], and Bonner [4] found a short lived intermediate, C678, which preceded C684. We report here that the C684 species can be transformed to C678 by light.

The observed photo- and dark transformations of P and C which occur *in vivo* can be summarized as follows:

$$P650 \xrightarrow{h\nu} C678 \underset{h\nu}{\overset{dark}{\rightleftharpoons}} C684 \xrightarrow{dark} C673 \xrightarrow{dark} C677$$

The light reactions are fast while the dark reactions are generally slow and temperature dependent.

2. Materials and methods

Dark-grown red kidney bean leaves were harvested after 9–13 days of growth at 24° [5]. All manipulations were performed under dim green safelight. Samples of four leaves were mounted between glass plates and placed in a massive thermostated brass block with optical ports in the sample compartment of a Cary Model 14 spectrophotometer. Actinic red irradiation was provided by a 500 W tungsten-halogen lamp with a heat and red plastic (Rohm and Haas 2444) filter. The intensity of the red irradiation at the leaf surface was 1.35×10^5 erg cm^{-2} sec^{-1}. About 25 sec of irradiation were required for full transformation of protochlorophyllide to chlorophyllide in the leaves with this source. Temperature was monitored by a thermocouple embedded in the leaves.

Absorbance spectra were scanned at 60 nm min^{-1} with a slit width of 1–1.5 mm. Between spectral measurements the measuring wavelength was set at 730 nm. No phototransformation was caused by the measuring beam under these conditions.

3. Results and discussion

The spectral shifts occurring following red irradiation of P650 and described by others [1–4] were observed. We confirmed that the initial product, C678, transforms rapidly at 3° to C684 with a marked temperature dependence and no isosbestic point.

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† *Abbreviations used:*
C: chlorophyllide;
P: protochlorophyllide.

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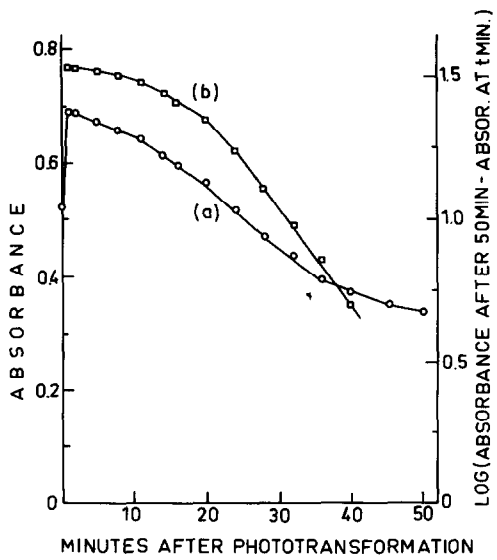

Fig. 1. Absorbance changes at 690 nm following phototransformations. Dark-grown bean leaves at 2° were irradiated with red light and their spectra recorded periodically following the initial irradiation. The initial rising portion of the curve (a) is a measure of the rapid C678 → C684 shift and the following declining portion is the C684 → C673 shift. Curve (b) is the log of the absorbance.

The dark conversion of C684 → 673 follows more slowly and has an isosbestic point at 676–677 nm. The C684 and C673 species have nearly equal absorbances at their respective maxima. The C684 → C673 transformation at 3° has first order kinetics following an initial lag phase (fig. 1b). The rate of transformation is leaf-age dependent, requiring 2 hr for completion in 15-day old leaves and 25 min for 8-day old leaves. The rate of transformation has a Q_{10} value of about 2 in the range of 2–25° which suggests that the reaction is chemical rather than physical.

We found that when leaves containing the C684 species are irradiated at 3° with red light, a C684 → C678 phototransformation is observed. Following the photoreversal to C678 by light, C678 undergoes a dark transformation to C684. This reaction can be repeated (fig. 2) at 3° but with diminishing yields with time due to partial irreversible losses of terminal species. This photoreaction is the only reversible step found in the last stages of chlorophyll biosynthesis. The photoreversibility of the reaction should provide a useful probe into the nature of the observed spectral shifts in chlorophyll biogenesis and plastid organization.

Under certain conditions, phototransformation of

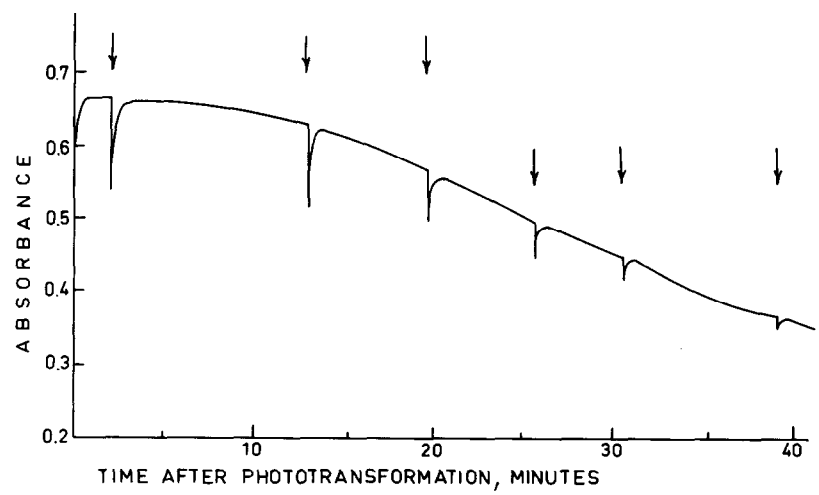

Fig. 2. Phototransformation of C684 → C678 and subsequent dark reversal. Dark-grown bean leaves at 2° were irradiated with red light to affect the P650 → C678 phototransformation. They were then irradiated for 30 sec periods with red light at times indicated by vertical arrows. The curve is the absorbance at 690 nm. The initial absorbance rise is due to the rapid dark C678 → C684 reaction and the overall slow decline is due to the slow C684 → C673 dark reaction. The rapid decrease and increase in absorbance is due to the C684 $\underset{\text{dark}}{\overset{h\nu}{\rightleftharpoons}}$ C678 light and dark reactions, respectively.

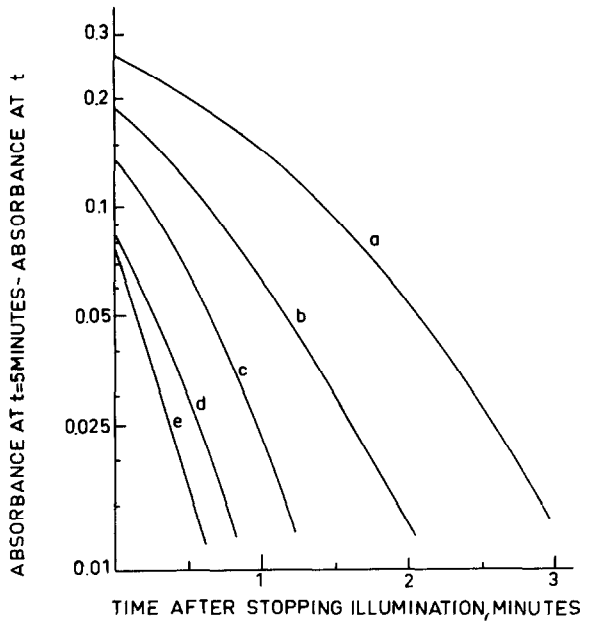

Fig. 3. The rates of the C678 → C684 rapid dark reaction following repeated irradiations. Dark-grown bean leaves at 2° were irradiated with red light to affect the P650 → C678 phototransformation. From the absorbance increase at 690 nm following 30 sec irradiations of red light, rates of the C678 → C684 rapid dark reaction were calculated. The order of illuminations is from a to e.

protochlorophyllide yields only C678 and it does not undergo transformation to C684. Examples are the C678 formed in phototransformation of isolated protochlorophyllide holochrome [5] and in frozen and thawed leaves [6]. Although a dark transformation of C678 to a shorter wavelength chlorophyllide is observed in these two cases, it cannot yet be determined if these transformations are similar to those observed in the intact leaf. If the leaves are frozen and thawed during the time C684 is present, photoreversal of C684 → C678 cannot be induced. Leaves treated with 2% glutaraldehyde at 3° show smaller and slower changes in spectral forms of chlorophyll but the C684 → C674 transformation persists. These observations suggest that some structural features of the developing chloroplast are required for the C684 → C677 photoreversal reaction and the C678 → C684 dark reaction.

The rate of the C678 → C684 dark reaction undergoes a change in kinetic characteristics with repetition of the C684 → C678 phototransformation. A family of curves is observed (fig. 3). Initially they appear to be complex, but then later they approach first order kinetics with increasing numbers of irradiation cycles.

The C678 derived from C684 by phototransformation and C684 from the dark transformation of C678 both slowly yield C673 as a dark reaction product. It is evident that the two C678 species are equivalent in all respects and are likely the identical species as shown in the initial scheme.

Acknowledgement

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References

[1] K. Shibata, J. Biochem. 44 (1957) 147.
[2] M. Gassman, S. Granick and D. Mauzerall, Biochem. Biophys. Res. Commun. 32 (1968) 295.
[3] C. Sironval, M. Brouers, J.M. Michel and Y. Kuiper, Photosynthetica 2 (1968) 268.
[4] B.A. Bonner, Plant Physiol. 44 (1969) 739.
[5] P. Schopfer and H.W. Siegelman, Plant Physiol. 43 (1968) 990.
[6] W.L. Butler and W.R. Briggs, Biochim. Biophys. Acta 112 (1966) 45.