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EVIDENCE FOR ENERGY TRANSFER FROM PROTOCHLOROPHYLLIDE TO CHLOROPHYLLIDE IN LEAVES TREATED WITH δ-AMINO-LEVULINIC ACID

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SUMMARY

From measurement of absorption and fluorescence spectra, at −196° we found that in δ-aminolevulinic acid (ALA)-treated leaves there is an important energy transfer from protochlorophyllide ALA absorbing at 649 nm, formed during the dark period following a first flash of light, to chlorophyll(ide) produced by this flash, at least at liquid nitrogen temperature. We also incidentally found that there is no accumulation of protochlorophyllide ALA absorbing at 649 nm at −5°. These facts are discussed in connection with the scheme proposed by Sundqvist.

INTRODUCTION

Etiolated bean leaves contain at least two forms of protochlorophyllide—lipoprotein complexes absorbing around 647 nm ($P_{657-647}$) and 629 nm, respectively; the corresponding fluorescence maxima are located around 657 and 631 nm. When an etiolated leaf is illuminated by a brief flash (≃ 1 msec) at room temperature, the absorption peak at 647 nm disappears and a new peak at 676 nm is found; this new form fluoresces at 688 nm ($P_{688-676}$); spectral shifts occur afterwards in the dark according to the scheme:

$$P_{657-647} \xrightarrow{h\nu} P_{688-676} \xrightarrow{\text{dark}} C_{696-682} \xrightarrow{\text{dark}} C_{680-672}$$

The position of the absorption and fluorescence maxima differ to a small extent according to the leaf material used [7,8,4]. From measurement of low-tem-

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Abbreviations: ALA, δ-aminolevulinic acid.

perature absorption, emission and excitation spectra of fluorescence of partly phototransformed bean leaves, evidence for energy transfer from protochlorophyllide to chlorophyllide was obtained [6,2].

Treatment of etiolated leaves with ALA causes accumulation of protochlorophyllide absorbing between 630 nm and 635 nm, and fluorescing from 633 to 640 nm ($P_{640-635}$ ALA).

A light flash of high intensity transforms the initial $P_{657-647}$ form into the initial $P_{688-676}$ form, but $P_{640-635}$ ALA remains unchanged.

During a subsequent dark period, a rapid regeneration of an absorption band at 649 nm ($P_{657-647}$ ALA) takes place. This regeneration is correlated with a simultaneous consumption of protochlorophyllide $P_{640-635}$ ALA [3,11]. A second high-intensity flash produces additional $P_{688-676}$ ALA from $P_{657-647}$ ALA.

MATERIAL AND METHODS

Leaf material and fluorescence spectra

Primary bean leaves (*Phaseolus vulgaris* L. cv. Commodore) grown in complete darkness were used; methods for growing the plants and for recording low temperature emission have been described elsewhere [9,1].

Sample irradiation

A 1-msec photographic flash (Multiblitz report Porba) was used. The leaves, placed at a distance of 5 cm from the lamp, were irradiated either at +22° or at −5°. A flash period consisted of two flashes separated by a 5-sec dark interval.

In vivo absorption spectra at −196°

The *in vivo* absorption spectra were recorded at liquid nitrogen temperature using a Cary 17 spectrophotometer. The leaf sample was placed in the measuring beam behind a 5 × 15 mm window cut in an adjustable copper plate, in a thick copper sample holder whose base was immersed in liquid nitrogen in a pyrex dewar. During cooling to −196°, a current of dry air circulated around the dewar to avoid water vapour condensing on the wall. A milk solution in water was used as a blank control to balance the diffusion effect of the leaf.

ALA treatment

8-day-old etiolated leaves were vacuum infiltrated in 30 ml of a 0.005 *M* ALA solution (Sigma Chemicals) in phosphate buffer 0.035 *M* (pH 6.5) for 3 h. Afterwards the leaves were spread for 15 h on filter paper in the upper part of a closed desiccator containing a little water in the bottom; under these conditions, the leaves are still turgid and pale green 48 h after the ALA treatment. All manipulations occurred in complete darkness or under a dim green safelight which has no effect on phototransformation.

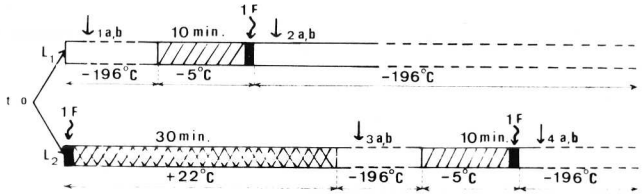

Fig. 1. Scheme of the manipulation: L_1 and L_2, two halves of an etiolated leaf treated with ALA; 1 F, one flash period.

RESULTS AND DISCUSSION

The following manipulation was made (Fig. 1): Two halves of the same ALA-fed leaf (cut along the midrib) were used (L_1 and L_2). The first half (L_1) was frozen in the dark at liquid nitrogen temperature. It was thereafter rewarmed in a thermostated room at −5° for 10 min, received a flash period at −5° (see METHODS) and was frozen again at −196° immediately after the flash period. The second half (L_2) received a first flash period at +22°, remained for 30 min in darkness at +22° and was then frozen at −196°. It was thereafter rewarmed at −5° for 10 min, received a second flash period at −5° and was frozen again in liquid nitrogen immediately after the second flash period.

Absorption and fluorescence spectra were registered during each period of cooling of the half leaves at liquid nitrogen temperature (arrows 1a, b; 2a, b; 3a, b; 4a, b in Fig. 1). It has been found that no change occurred in the absorption and fluorescence spectra registered at −196° when an etiolated leaf was frozen at −196°, rewarmed at −5° for 30 min and refrozen at −196° in complete darkness. It was also seen that no accumulation of $P_{657-647}$ ALA occurred at −5° within 30 min after a flash period (Fig. 2).

This is consistent with the results of Granik and Gassman [5]. Some enzyme process is apparently required for generation of $P_{657-647}$ ALA.

Absorption and fluorescence spectra in Fig. 3 show that:

(*1*) A 30-min dark period at +22°, following a first flash period, was sufficient to allow the formation of the same amount of $P_{657-647}$ ALA, as the amount of $P_{657-647}$ initially present in the (ALA-fed) leaf before the flash period (curve 3a). The absorption increase in the 650 nm region, was accompanied by a corresponding decrease in the 636 nm region (compare curve 1a to 3a). The generated protochlorophyllide ($P_{657-647}$ ALA) was transformed into chlorophyllide by a second flash period (curve 4a).

(*2*) During the 30-min dark period at +22°, after a first flash period, a shift of the chlorophyll(ide) absorption band from 676 to 671 nm occurred (fluorescence shift from 688 to 685 nm; compare curves 2a and 3a).

(*3*) Only a small shoulder was seen at 657 nm in the emission spectrum after the 30-min dark period at +22° which followed a first flash period (curve 3b), although an important absorption peak was seen after that dark period at

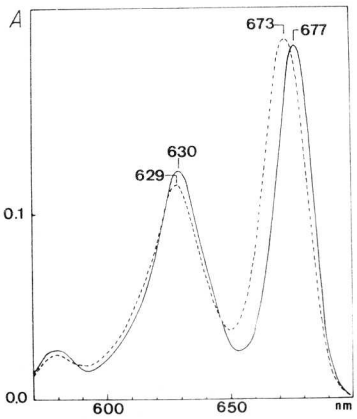

Fig. 2. Absorption spectrum of ALA treated leaf at −196°: (——), first half of the leaf frozen at −196° immediately after a first flash period at −5°; (— — —), second half of the leaf which received a first flash period at −5°, was warmed for 30 min in darkness at −5° and was frozen immediately at −196° after that time.

649 nm (curve 3a). The fluorescence intensity in the 685 nm region was increased and the emission band was enlarged after the 30-min dark period.

A second flash period did not markedly change the emitted fluorescence,

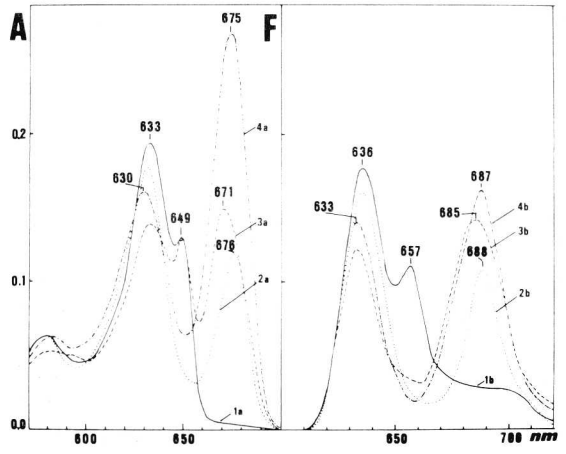

Fig. 3. Change in absorption (a) and fluorescence (b) spectra at −196° in ALA-treated leaves upon illumination: 1a, b, first half of the leaf etiolated; 2a, b, first half of the leaf immediately after a flash period at −5°; 3a, b, second half of the leaf 30 min after a first flash period at +22°; 4a, b, second half of the leaf immediately after a second flash period at −5°. The fluorescence spectra are corrected for energy variation in the photomultiplier response. The ratio between the heights of the 636 nm fluorescence peaks are adjusted to the ratio between the corresponding 633 nm absorption peaks.

although it considerably changed the absorption of the leaf (compare 4b to 3b and 4a to 3a). This clearly indicates an energy transfer from generated protochlorophyllide $P_{657-647}$ ALA to chlorophyll(ide) molecules formed by the first flash period, the efficiency of which may approach 100%.

Energy transfer from $P_{657-647}$ ALA to chlorophyll(ide) makes it plausible that the chlorophyll(ide) molecules produced as the result of the absorption of a first flash, remain in close proximity to $P_{657-647}$ ALA molecules accumulating thereafter in darkness, even after the Shibata shift had been completed. This is in partial contradiction with the scheme proposed by Sundqvist [11,12], after which chlorophyll(ide) moves apart, or leaves the protein moiety of the pigment–protein complex after irradiation. The facts require a change in the protein–pigment relationship such that, after having moved from the active site, the chlorophyll(ide) molecules produced by a first flash, remain in close connection with the new (ALA) protochlorophyllide molecules which reach the empty sites in darkness ($P_{657-647}$ ALA).

Sironval and Kuiper [10] proposed a model where the prolamellar body is described as being composed of "energy-transfer units" containing n pigment molecules linked to a lipoprotein. After light absorption, (n–m) among these molecules still absorb in the red at 647 nm but transfer energy inside the unit to m molecules having a red absorption band at 676–678 nm. Any given 676–678 nm pigment molecule emits a 688 nm fluorescence from energy collected inside the unit to which it belongs. At ordinary temperature and in the leaf, the transfer unit contains about 18 pigment molecules. Since there is no energy transfer from $P_{640-635}$ ALA protochlorophyllide molecules to chlorophyll(ide) molecules produced by a first flash of light, it is concluded that $P_{640-635}$ ALA does not belong to the transfer units as defined by Sironval and Kuiper [10]. On the contrary, the occurrence of an energy transfer to chlorophyllide from ALA protochlorophyllide molecules after having reached the empty sites ($P_{657-647}$ ALA) shows that these molecules necessarily enter the transfer unit. These units are still present even after the Shibata shift has been completed.

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