A RAPID SPECTRAL CHANGE IN ETIOLATED RED KIDNEY BEAN LEAVES FOLLOWING PHOTOTRANSFORMATION OF PROTOCHLOROPHYLLIDE[1,2]

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When leaves of dark-grown angiosperm seedlings are exposed to light, the protochlorophyllide-protein complex (holochrome), absorbing at 650 nm *in vivo*, is reduced to chlorophyllide absorbing at 683-684 nm (Shibata, 1957; Butler, 1961; Sironval *et al.*, 1965). During an ensuing 10 to 30 minutes, the absorption maximum shifts from 683 nm to 672 nm (Shibata, 1957) while the chlorophyllide is esterified with phytol to chlorophyll (Sironval *et al.*, 1965).

We have found that the shift of 650 nm to 683 nm consists of two separate steps. The first step is the photoconversion of the protochlorophyllide with a maximum at 650 (P_{650}) to chlorophyllide with absorption maximum at 678 (C_{678}). This is complete within 1 second at room temperature. The second step is a slower shift (30 seconds at room temperature in 9 day-old leaves) of the 678 nm maximum to a 683 nm maximum. Evidence is presented that this second step represents a physical, environmental change of the newly formed chlorophyllide.

MATERIALS AND METHODS

Red Kidney beans were germinated in the dark for about 9 days at about 22°. The absorption spectra through four thicknesses of primary leaf tissue were measured in a Cary Model 14 Spectrophotometer against an appropriate scattering material in the reference cuvette. Phototransformation of the protochlorophyllide was accomplished with either electronic or regular flash bulbs, the emitted light being reflected through the leaves by a half-silvered mirror. The time constant for detection of absorbancy changes was one second with a delay of 5 seconds between the flash and first measurement.

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Changes in fluorescence emission were measured on a single layer of leaf tissue, the protochlorophyllide being photoconverted with the same beam used to excite fluorescence. The time delay for detection of fluorescence changes was less than 10 seconds, using a Hitachi Perkin-Elmer Model MPF-2A Fluorescence Spectrophotometer.

RESULTS

The dark-grown leaf, after an actinic flash, forms nascent chlorophyllide with an absorption maximum at 678 nm. This maximum is shifted in the dark to 683 nm within 30 seconds after the flash at room temperature in 9 day-old leaves.

To study the shift more readily, the time course of the change was prolonged by decreasing the leaf temperature to 0° and by using older seedlings (13 days) (Figure 1). With no illumination (curve A) the 650 nm maximum of protochlorophyllide holochrome is evident. Some 30 to 90 seconds after a flash of light (curve B), the absorption band at 650 nm has diminished and, concomittantly, the chlorophyllide band maximum at 678 nm appears. Five minutes after the flash (curve C), the chlorophyllide maximum has shifted to 683-684 nm. The shift from 678 nm to 683 nm does not possess an isosbestic point.

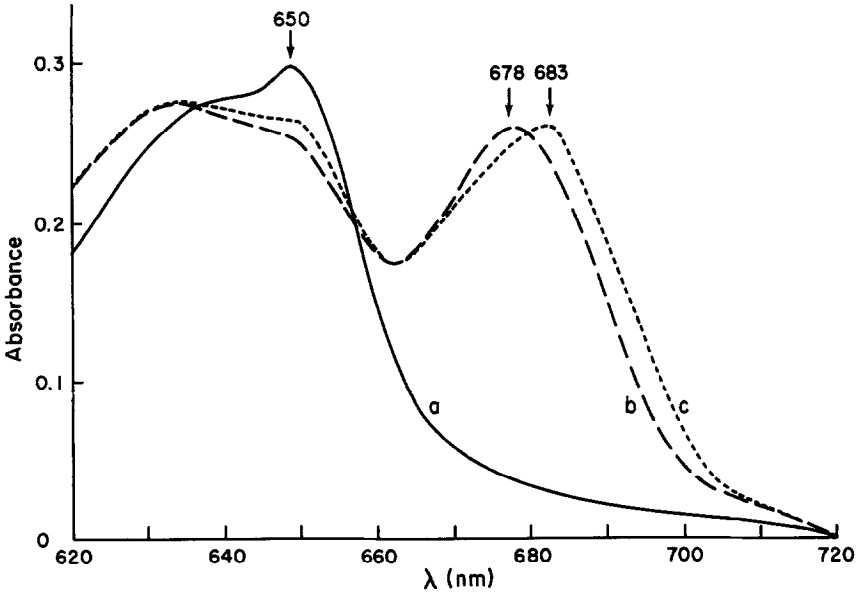

Figure 1

In vivo spectra of 13 day-old etiolated Red Kidney bean leaves (4 tissue layers); (a) no illumination; (b) 30 to 90 seconds following a flash; (c) 5 minutes after the flash.

Temperature 0°C.

To better measure the early time course of the shift, advantage was taken of the fact that the maximum absorbancy difference between the 678 and 683 curves occurs at 692 nm. By monitoring the change in absorbancy at 692 nm with time after a flash of light, the first change can be detected within 5 seconds. The curve so obtained is shown in Figure 2.

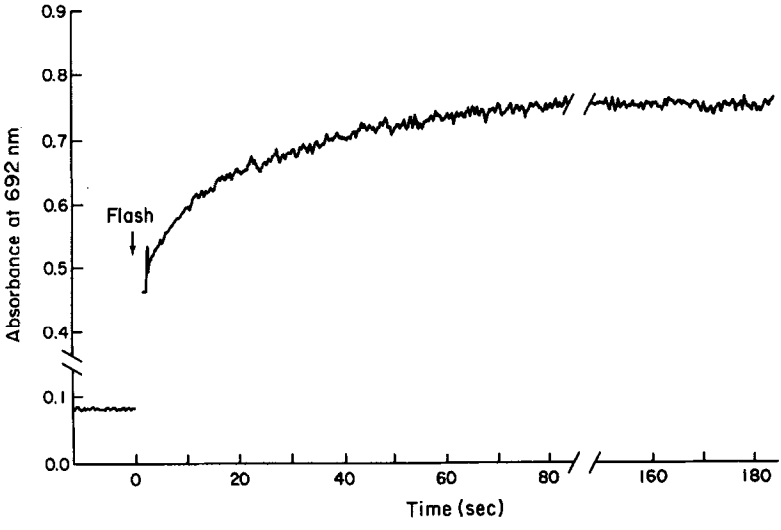

Figure 2

The change of absorbance at 692.5 nm (the point of maximum absorbancy change when 678 is converted to 683) with time following a flash. Temperature 14°C, 9 day-old leaves.

Changes in the fluorescence emission of the etiolated leaf after excitation with 445 nm light have been studied. A fluorescence peak appears at 685 nm within 10 seconds after the leaf is exposed to the excitation beam; this represents nascent chlorophyllide (C_{678}) as seen by absorption spectra. The peak then decreases in height and broadens in a time-dependent and temperature-dependent manner comparable to the 678-to-683 shift observed in the absorption spectrum measurements. This broad emission peak increases in intensity and exhibits a maximum at 677 nm during a 7 to 25 minute period following phototransformation. This last change corresponds to the 683 nm-to-672 nm absorption change (cf. Butler, 1961).

The following conditions affected the rate and nature of change of the shift from 678 to 683 nm absorption maxima:

1. Freezing. At -70°, the photoconversion of protochlorophyllide (P_{650})

to chlorophyllide (C_{678}) occurs, but the shift to chlorophyllide (C_{683}) is absent (cf. Butler and Briggs, 1966).

2. Temperatures above 0°. At temperatures above 0° the shift is the more rapid, the higher the temperature. A Q_{10} of about 2.5 is observed between 1° and 22° (Figure 3).

3. Glycerol effect. Infiltration of the etiolated leaves in the dark for 6 hours with 30% glycerol prolonged the time course of the chlorophyllide shift. At 26° the change requires 120 seconds in 6 day-old leaves as compared to 10 seconds in controls.

4. Leaf age. Fourteen day-old leaves require 135 seconds to complete the shift at 12° as compared to 70 seconds in 7 day-old leaves.

5. Freezing and thawing dark-grown leaves diminishes the absorption peak at 650 nm and increases the absorbancy at 633-637 nm (cf. Butler and Briggs, 1966). Illumination of these leaves produces chlorophyllide (C_{678}) but the shift is to 672 nm instead of 683 nm.

6. Infiltration of dark-grown leaves with 0.01M δ-aminolevulinic acid (ALA) for 8 hours produces large amounts of inactive protochlorophyllide (P_{633}). Illumination of such leaves produces chlorophyllide (C_{678}) but the shift is to 675 nm instead of 683 nm.

DISCUSSION

Evidence has been presented that a shift from 678 to 683 nm of the nascent chlorophyllide takes place rapidly in the leaf. This shift is best interpreted as indicating that nascent chlorophyllide undergoes environmental changes shortly after being formed. As shown by Gassman and Bogorad (1967), the protochlorophyllide holochrome behaves as an enzyme. Thus, one may consider that chlorophyllide is the product and its release from the holochrome is a slow step. The rather high temperature coefficients (Figure 3) of this shift suggests that the binding to, and thus the rate of release of the chlorophyllide from the holochrome and other sites, requires appreciable energy. Bogorad et al. (1968) report that the photoreduction of protochlorophyllide holochrome in the leaf or in a preparation of holochrome isolated from the leaf leads to the formation of a fragment of low buoyant density containing the chlorophyllide. They suggest that this small fragment may serve as an intermediate carrier for the chlorophyllide.

When the change in absorbancy with time after a flash is observed, as in Figure 2, at wavelengths between 679 nm and 682 nm, the shape of the curve is different from that seen at other wavelengths. The absorbance at a given wavelength in this interval passes through a maximum as the absorption peak changes from 678 nm to 683 nm. This suggests that the chlorophyllide passes through

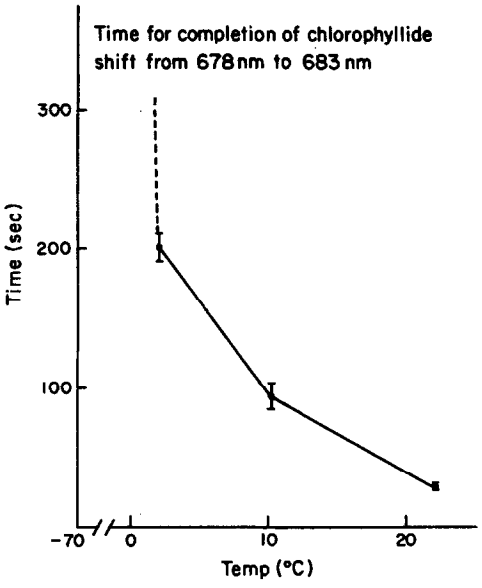

Figure 3

The time required for completion of the chlorophyllide shift from 678 nm to 683 nm in 9 day-old bean leaves at various temperatures. The shift is not observed in frozen leaves.

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more than one change of environment during this time period.

The decrease of fluorescence on forming chlorophyllide (C_{683}) suggests that the chlorophyllide is aggregated, as proposed by Butler and Briggs (1966). Furthermore, the observation here that the wavelength shift is from 678 nm to 675 nm, rather than to 683 nm, in the presence of excess inactive protochlorophyllide (P_{633}) suggests that the chlorophyllide is aggregating with the excess protochlorophyllide rather than with itself. The relatively long wavelength of the protochlorophyllide holochrome (650 nm) and of the chlorophyllide at 683 nm may be caused by aggregation. Butler and Briggs (1966) and Seliskar and Ke (1968) have suggested that protochlorophyllide (P_{650}) may be an aggregated form of protochlorophyllide.

These facts suggest the following interpretation: After photoconversion, chlorophyllide (C_{678}) would have to diffuse from the holochrome, in order to permit the protein to act again as a photochemical "enzyme". Several intermediate steps appear to be involved: one being a diffusion of the chlorophyllide from the holochrome protein, perhaps on a carrier, a second being an aggregation of the pigments, a third possibly being the localization of the chlorophyllide (C_{683}) on a lipoprotein complex where phytylation and membrane synthesis can occur.

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