FLUORESCENCE AND PHOTOTRANSFORMATION OF PROTOCHLOROPHYLL WITH ETIOLATED BEAN LEAVES FROM -196 TO + 20°C

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Summary

The fluorescence yield of convertible protochlorophyll(ide) decreases markedly between -196 and -90°C, while the decline of fluorescence yield of chlorophyll(ide) and non-convertible protochlorophyll is much less pronounced. A comparison of temperature dependence of fluorescence of etiolated leaves with fluorescence of photosynthetically active chloroplasts shows a similarity of the F656 protochlorophyll band in the former, with the F695 chlorophyll *a* band in the latter. A dark transformation after illumination can be measured only between -90 and -40°C, both with intact leaves and leaf homogenates.

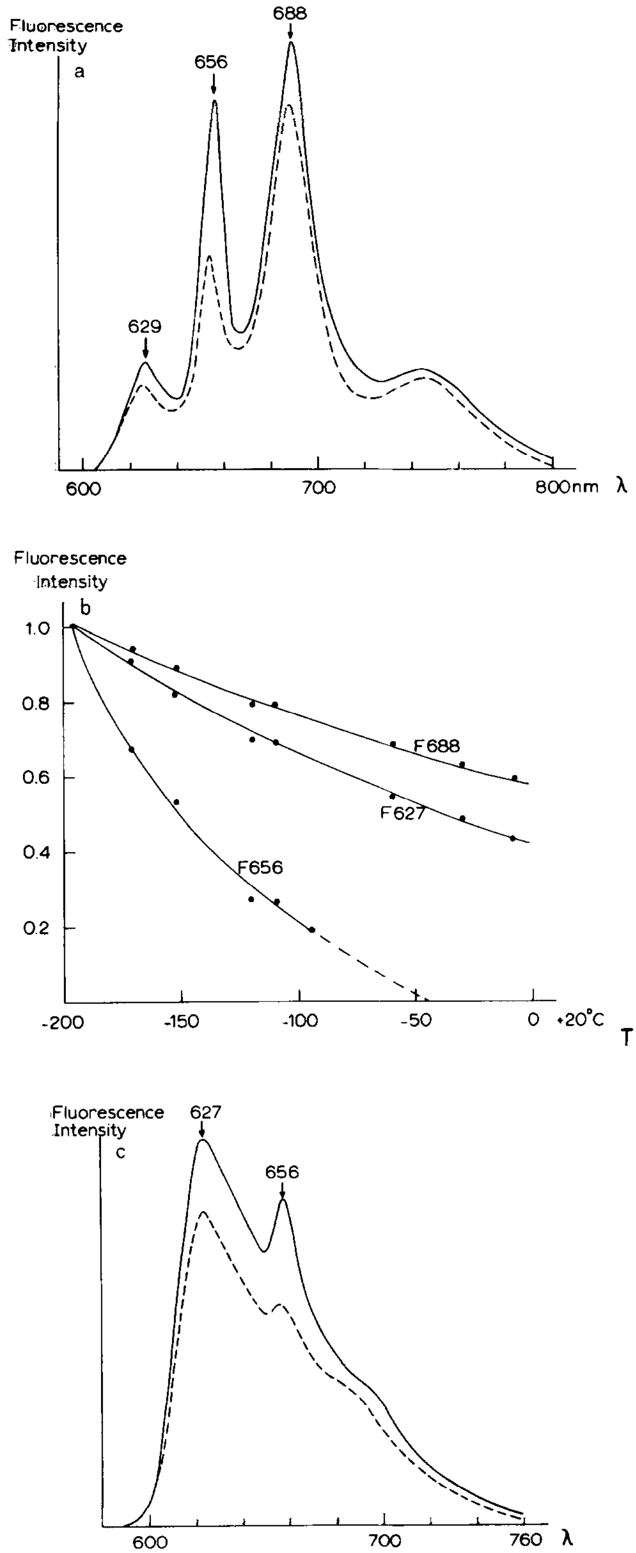
Protochlorophyll(ide) in etiolated bean leaves in converted into chlorophyll(ide) at room temperature with a high quantum efficiency[1]. Hence a fluorescence spectrum of etiolated leaves containing convertible protochlorophyll cannot easily be measured at this temperature. No transformation, however, occurs if illumination is done at temperatures below -80°C[2]. Consequently the fluorescence spectrum of protochlorophyll in vivo can be measured at liquid nitrogen temperature[3][4][5].

In the fluorescence spectrum of etiolated leaves measured at this temperature, bands are found at 629, 656 and 710 nm. If the leaf is illuminated at -40°C, part of the protochlorophyll bands can be transformed into chlorophyll bands with maxima at 688 and 745 nm. In Fig 1a a fluorescence spectrum of such a leaf is given, illuminated with mainly 437 nm Hg light. The spectrum is measured at -196°C and, after removal of N_2 and warming up the sample in the dark, at -100°C.

It follows from the figure that the fluorescence band at 656 nm of convertible protochlorophyll has a higher temperature coefficient than the chlorophyll F688 and the F629 band. The latter one is often assumed to be emitted by non-convertibe protochlorophyll. Fig.1b shows that extrapolation of F656 temperature dependence leads to very low values around room temperature.

Experiments with chlorophylls in vitro showed that absorption bands are sharpened and shifted towards longer wavelength as a result of cooling[6]. Hence a decrease in fluorescence might be produced by a decrease in absorption of Hg light. Therefore the experiments were repeated with an incandescent lamp(provided with a 3 cm 6% $CuSO_4$ filter to remove far red light) which gives a continuous spectrum. As the surface under the absorption curve (transition probability) is not assumed to change markedly during cooling, fluorescence excited with this lamp will be little affected by sharpening and shifting, provided the fluorescence yield does not change. As the curves in Fig. 1b did not change markedly, the decrease in fluorescence intensity indeed is caused by a decrease in yield. A decrease in yield can indicate a decrease in mean lifetime of fluorescence. Such a decrease (from 5.10^{-9} to 2.10^{-9} sec) indeed was measured when etiolated leaves were warmed from -196° to -100° by Rubin et al.[7]. If all convertible protochlorophyll is transformed before temperature dependence is measured, the curves of F688 and F629 can be extended to room temperature.

The rate of phototransformation, measured by increase in F688, was found to be highly temperature dependent. From -90 to -70°C an 8-fold increase occurs, from -40 to -20° a 2-fold increase. As was measured after refreezing to -196°C, prolonged illumination at -80°C results in a disappearence of more than 95% of F656. This suggests that in bean leaves nearly all convertible protochlorophyll(ide) can be transformed at -80°C, be it that the rate of transformation is low. This result is in contrast to the findings of Smith and Benitez[2], who found that with barley


Fluorescence Intensity
a
688
656
629
600
700
800nm λ
Fluorescence Intensity
b
1.0
0.8
0.6
0.4
0.2
F688
F627
F656
-200
-150
-100
-50
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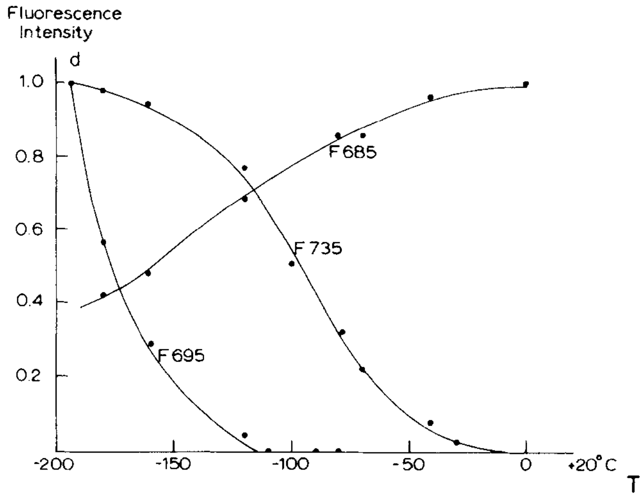

Figure 1 a. Fluorescence spectrum of an etiolated bean leaf, which has been illuminated at -40°C until about half of the protochlorophyll(ide) band at 656 nm is converted into a chlorophyll(ide) band at 688 nm. Fluorescence is excited by light mainly of 437 nm, and measured at -196°C(---) and -100°C(- - -).

b. Fluorescence intensity of F656, F688 and F629 at different temperatures after warming from -196°C.

c. Fluorescence spectrum of an etiolated bean cotyledon from the same plant. The spectrum is measured at -196° (---) and -100°(- - -).

d. Fluorescence values in the maxima of F685, F695 and F735 with chloroplasts of Spinacea oleata at various temperatures between -196 and 0°C. A correction for overlap of the different fluorescence bands is applied. This correction results from measurements with chlorophyll a in vitro at different temperatures. All values are put at 100 at the maximum of the curve. The shape of F685 depends to a large extent on the amount of F695 in the sample studied.

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leaves at -60°C only 40% of protochlorophyll can be transformed by illumination.

Around -80°C a dark transformation after illumination can be measured. As shown in Fig. 2, the increase of the F688 chlorophyll band proceeds after

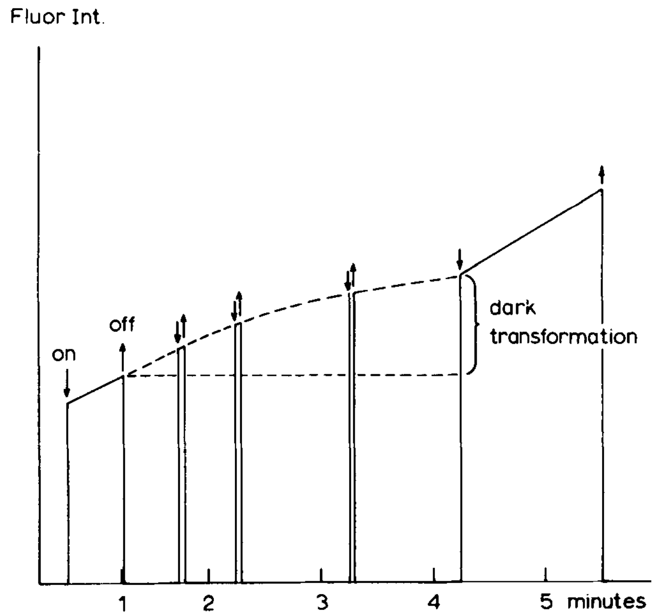

Figure 2 Increase in 688 nm fluorescence, assumed to indicate chlorophyll(ide) a formation, both in the light and in the dark after end of illumination. The curve is measured at temperatures between -80 and -75°C. Short light pulses (0.5 sec) were used to measure the shape of the dark transformation curve. Approximately the same end value is reached if a period of complete darkness is applied. No dark transformation was found above -40°C, or if illumination had occurred below -120°C.

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illumination is ended. The effect could be repeated after a few minutes renewed illumination. The dark transformation occurs much faster at higher temperatures, and could not be measured above -40°C. At room temperature the time constant of a dark transformation should be shorter than 4 msec[8]. The temperature dependence of the dark transformation in the protochlorophyll-chlorophyll conversion in bean leaves thus is quite different from that encountered with enzymatically driven processes. The effect might be related to the physical state of bound water in the holochrome protein.

A similar, even more pronounced, dark transformation is seen when a filtered homogenate of etiolated leaves is used in the experiments.

No dark transformation was observed when an etiolated leaf was illuminated between -196 and -120°C, warmed to -40°, kept at this temperature for 15 minutes and recooled to -196°C. This indicates that light absorbed at very low temperatures is not used in the dark reaction.

If the leave is allowed to thaw in the dark after freezing with liquid N_2 a small fraction of protochlorophyll becomes non-convertible[9], while thawing a leaf which has been frozen slightly below 0°C results in inactivation of most of protochlorophyll[1][2]. The non-convertible fraction was found to have fluorescence maxima at 635 and 690 nm. A similar spectrum is observed if inactivation occurs as a result of heating to 55°C. After freezing to -196°C, thawing and refreezing, the fluorescence of the inactive fraction fills in the minimum between the F629 and F656 bands, thereby slightly distorting the fluorescence spectrum. The shape of the F629 band in the fluorescence spectrum of intact bean leaves differs from that of non-convertible protochlorophyll, produced by heating and thawing. This may be seen clearly in cotyledons of etiolated bean plants, frozen in the dark. Here the F629 band, located in cotyledons at 627 nm, is often higher than the F656 band. As F629 has a much lower temperature coefficient than F656, the latter one is weak at -95°, and the total fluorescence spectrum at this temperature is mainly due to F629 (Figure 1c).

The increase in fluorescence yield of active protochlorophyll (F656), measured when the sample is cooled from -90 to -196°, resembles the increase in F695 fluorescence in photosynthetically actice cells or chloroplasts of chlorophyll a containing organisms. When such preparations are cooled to -196°, two new fluorescence maxima at about 695 and 717-740 nm are observed besides the band at 685 nm seen at room temperature. Warming to -120° results in a disappearence of F695, while F735 decreases only gradually[12]. Recooling was found by us to result in a reappearence of the 3-topped spectrum. In Fig. 1d the temperature dependency of F685, F695 and F735 of spinach chloroplasts is given. In these chloroplasts all bands are nicely separated. For an estimation of the correction due to overlap of the different fluorescence bands, spectra of chromatographed chlorophyll a in a glycerol/phosphate (pH 7.3) mixture to which 0.5 % Triton X-100 was added, were determined at temperatures down to -196°C. Such a mixture freezes as a clear glass[12], and exhibits a high fluorescence yield. The curves of Fig. 1d can be repeated several times by successive cooling and warming, provided the temperature does not exceed about -40°C.

The similarity in temperature dependency of F695 in chloroplasts and F656 of convertible protochlorophyll suggests a structural similarity immediately around the emitting pigment molecule.

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