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PHYTOCHROME DECAY AND REVERSAL IN LEAVES AND STEM SECTIONS OF ETIOLATED PEA SEEDLINGS

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INTRODUCTION

In many plants, photoconversion of P_r to P_{fr} is followed in the dark by a gradual decrease in total phytochrome (1, 2), whereas in others, a reversal to the red absorbing form could be demonstrated (2, 3). Absorption spectrum changes during such reactions in pea plumules pointed to the occurrence of several reactions, one of them leading to the formation of a pigment with maximum absorption at 650 nm (4). In the mean time, we have found that the photoreversible pigments in leaves of some etiolated plants apparently differ from those, present in the stems (5). We have, therefore, reinvestigated the reactions mentioned above with pea leaves and internode sections separately. In additions, we have paid more attention to the oxygen requirements of the decay reaction (6). In this paper we will distinguish between phytochrome 'decay', defined as disappearance of the 730 nm absorption band without concomitant increase in optical density in the red region, and phytochrome 'reversal', defined as disappearance of 730 nm absorption with formation of an equivalent amount of optical density in the red.

METHODS

Most technical details have been given previously (4). The material described there as plumules, consisted of the primary leaves with about 7 mm of the stem (third internode) attached to them. We have now taken care to separate the two parts as completely as possible. As this had to be done in complete darkness, it was unavoidable that a fraction of the leaves collected still had a small piece of stem on them.

Absorption cells were made of perspex and had a depth of 6 mm. Through an inlet at the bottom a slow stream of gas could be passed through the samples.

Aerobic atmospheres were either air or pure oxygen. For anaerobic conditions, ultra pure argon was passed through. All gasses were thoroughly humidified before entering the absorption cells.

Two absorption cells were filled with plant material. Both samples were irradiated with about 2×10^7 erg/cm^2 red (653 nm). Subsequently, one cell got an additional dose of about 7×10^6 erg/cm^2 far red (737 nm) to reconvert the phytochrome into the stable red absorbing form. This cell usually was placed in the reference beam of the scattered transmission accessory of the Cary-14 spectrophotometer. The other cell, with the phytochrome in the far red absorbing form was placed in the front beam. If considered necessary, it could be irradiated another time at any moment without being removed from the spectrophotometer. Thirty minutes after the actinic irradiations, a recording of the spectrum was started. This served as the reference base line (time zero). After certain intervals, the spectrum was recorded again, the density differences with the first recording providing the difference spectra of the changes that had taken place during the preceding period. In the interval between two spectrum runs, the sample cells were protected from the measuring beams by closing the spectrometer slits.

The temperature in the cell compartment during the measurements was $25° \pm 1$ °C.

One gram lots of pea leaves, harvested in complete darkness were subjected to the same treatments as applied during the spectrum measurements. After appropriate dark periods, the samples, together with a blank that had been kept in the dark throughout, were extracted with 80% acetone. The extraction was repeated twice. After making up to volume, protochlorophyll in the samples was measured by a method, similar to the one given by BOARDMAN (11).

RESULTS, DISCUSSION

In the presence of sufficient oxygen, P_{fr} mainly undergoes decay, both in leaves and in internode sections, no appreciable red absorption being formed (Fig. 1). The small positive absorption changes may partly be accounted for as due to some reversion of undecomposed P_{fr} competing with the decay reaction. In addition, most samples showed relatively large absorption changes towards the violet. These seem to be unspecific, of variable sign and magnitude, and are probably not related to the phytochrome conversions as such. Results such as those of fig. 1 give rise to the conclusion that, if we disregard the small absorption changes below about 670 nm, the spectrum essentially represents the negative of the absorption band of P_{fr} and that the latter pigment is converted during the decay reaction into substances having no appreciable absorption between 500 and 800 nm.

A further conclusion is that the ratio $O.D._{665}/O.D._{730}$ in the absorption spectrum of P_{fr} approaches zero, i.e. that the absorption coefficient of this pigment at 665 nm is very low. This forms a contrast with values, derived from absorption and action spectra of purified phytochrome preparation (7) which yield values for this ratio of about 0.29 and 0.25, resp. On

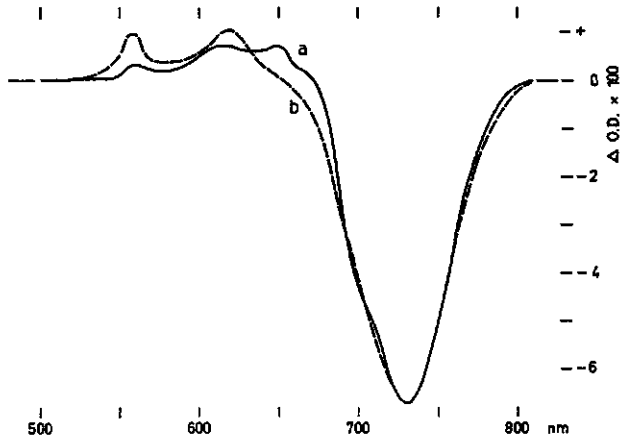

FIG. 1. Difference spectra for the dark reactions in the presence of oxygen.
a. Isolated pea leaves,
b. sections of third internode.

the other hand a value of less than 0.05, as found now, is in harmony with the form of the absorption spectrum of P_{fr} as derived from the action spectra (8) and the spectra for photoconversion of the pigment at low temperature (9). It seems possible, therefore, that the absorption spectrum of P_{fr} does not extends as far towards shorter wavelengths as is suggested by the absorption spectra of purified pigment extracts. On the other hand, P_{fr} certainly must have some absorption at 650 nm. This is demonstrated by the following, at first sight somewhat surprising, observation. Illumination of P_{fr} at low temperature (–196°) with red (653 nm) bleaches the 744 nm absorption band as much as does far red, although, of course, a larger dose of red is required. See also the action spectrum for this reaction at –70° (ref. 8, fig. 2f).

Difference spectra for dark transformation in the absence of oxygen show a slow disappearance of the absorption band of P_{fr} accompanied by development of an absorption band in the red region, fig. 2. A comparison of these spectra with those for the phototransformation of P_{fr} following irradiation with far red light shows that the preponderant reaction in this case is pigment reversal, fig. 3 and 4. Further, the different nature of the pigments in leaves and stem sections is also reflected clearly in the dark reversal spectra. This forms an additio-

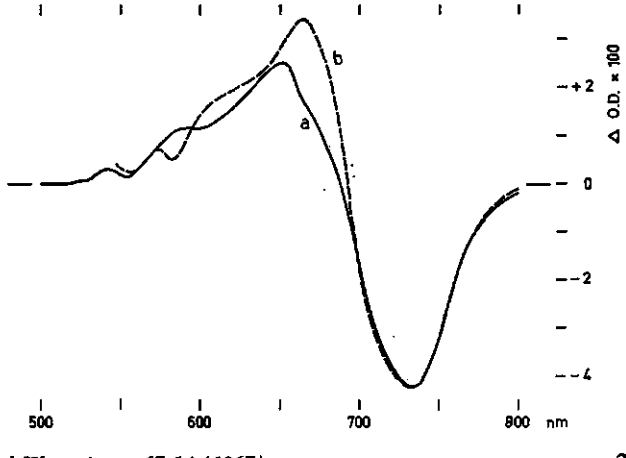

FIG. 2. Difference spectra for the dark reactions under anaerobic conditions.
a. Isolated pea leaves,
b. sections of third internode.

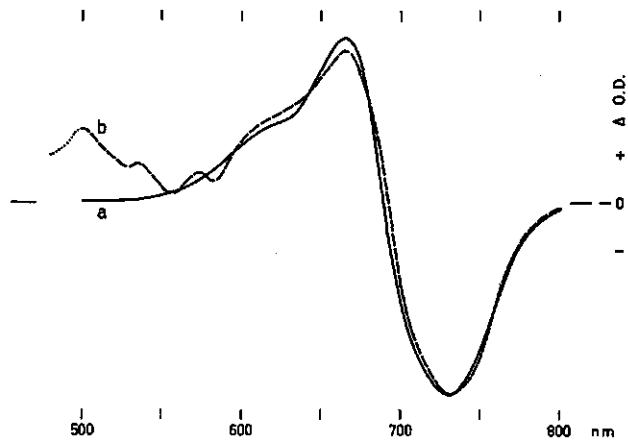

FIG. 3. Comparison of difference spectra for the reactions in sections of third internode.
a. Phototransformations upon far red irradiation, b. anaerobic dark transformations.

nal support for the conclusion that phytochrome in pea leaves differs from the pigment in the stems.

The difference spectrum for the dark transformation in mixed leaf-internode samples, published previously (4) can now be compared with those of the separate organs as shown in fig. 1. Evidently, the former was almost exclusively due to the leaves. This is in agreement with the observation that, in general, the reactions in the internode sections are much slower than those in the leaves. In both respects, the agreement between the present experiments and those of 1965 is satisfactory. There are, however, serious discrepancies as far as the rates of the decay and reversal reactions are concerned, see table I.

TABLE I. Rate of disappearance of 730 nm absorption band. $t_{\frac{1}{2}}$ (minutes)

Gas phase	Leaf	Type	Internode	Type	Mixed (Expts. '65)	Type
argon	222–292	R	240–417	R		
not aerated	75	D	675	R	60	R
air	85–100	D	130–168	D		
oxygen	60	D	80	D		

Type R: difference spectra of the reversal type (fig. 2)
Type D: difference spectra of the decay type (fig. 1)
The times indicated are those, required for the disappearance of one half of the optical density at 730 mm, generated by the initial red actinic irradiation.

The rates are rather variable even for the same type of material. Nevertheless, it seems safe to conclude that phytochrome decay is considerably more rapid than reversal. There is, however, a serious disagreement with the experiments of 1965 in that the latter, though having a reversal type spectrum, showed much higher reaction rates than were now observed. In the present experiments without forced aeration, these rates were duplicated, but the reaction was of the

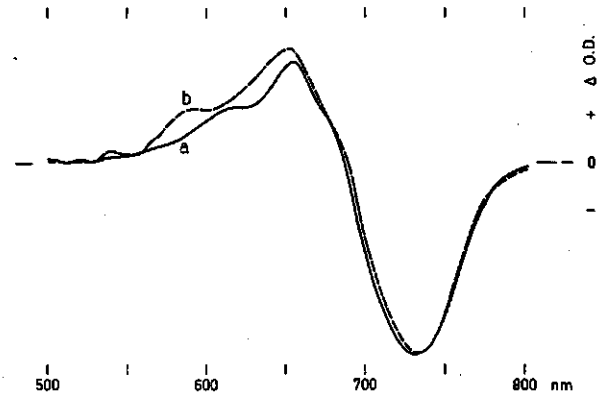

FIG. 4. Comparison of difference spectra for the reactions in isolated pea leaves. a. Phototransformations upon far red irradiation, b. anaerobic dark transformations.

wrong type. As a matter of fact, one would not expect much decay in this type of experiment, as the relatively small quantity of oxygen in the sample vessels certainly must have been exhausted by respiration in a time, short compared with the duration of the measurement, so that, essentially, the conditions would be anaerobic. At any rate, it seems that the respiratory capacity of the material may be important for the course and speed of the dark reactions. Not much more can be said about this point at the moment.

Finally, a few words must be devoted to the role of protochlorophyll in these reactions. Previously (4) we have attempted to attribute the 650 nm band formed during the dark reaction in pea plumules to formation of protochlorophyll on the basis of the position of the absorption maximum and on the known stimulation of protochlorophyll resynthesis by red illumination and its reversal by far red (10). Apparently, this conclusion was supported by the difference spectrum for a repeated irradiation of the sample at the end of the dark reversion period (see ref. 4, fig. 2). This spectrum shows beyond reasonable doubt that a lot of protochlorophyll must have been formed during the dark period. Now, we have to admit that this conclusion, though valid in itself, is not relevant to the interpretation of the dark reversion spectra. This is, because the difference spectra represent the difference in light absorption between two similar samples, one of which serves as a reference. At the start this reference vessel had obtained the same dose of red as the sample vessel, followed, however, by far red. During the second red illumination at the end of the measurement, this vessel was kept in the dark, however. Therefore, our conclusion as to the formation of protochlorophyll, though correct, can only explain the observed spectra as long as the rate of protochlorophyll formation in the reference vessel was much lower than that in the sample vessel. This, however, was not tested for our material. To check this, we have now determined in separate experiments the quantities of protochlorophyll in samples of pea leaves, subjected to the same treatment as applied during the measurement of the reversal and decay spectra. Within the limits of error, the amount of protochlorophyll formed after four hours of darkness was the same for material that had been given red only as for material that had been irradiated with red followed by far red. We cannot, in this case, find a reversing action of far red upon induction of protochlorophyll resynthesis by red. The significance of this finding for our present problem depends upon two factors: the magnitude of the error in protochlorophyll estimation, and the relative contributions of protochlorophyll and P_r to the absorption at 650 nm. This can be estimated from spectra such as fig. 2, ref. 4, and the ratio *O.D.* protochlorophyll/*O.D.* P_r at 650 nm turns out to be about 0.8. If we allow for an error in the estimation of protochlorophyll of 10%, at most 8% of the absorption changes at 650 nm can be attributed to differential protochlorophyll resynthesis in

the two vessels. We must, therefore, conclude that the 650 mn absorption band in the reversal spectra is indeed due to a pigment that is not protochlorophyll. It remains possible that the weak band at 650 nm in the decay spectra, fig. 1, is partly due to differential protochlorophyll resynthesis.

An abstract of this work was presented elsewhere (12).

Summary

Spectral changes during a dark period following red irradiation of primary leaves and sections of third internode of dark grown pea seedlings have been studied. In the presence of sufficient oxygen, in both organs the absorption band of P_{fr} at 730 nm disappears without formation of appreciable absorption in the red. Under these conditions, P_{fr} is, therefore, transformed into products not absorbing to any large extent in the region 500–800 nm. On the other hand, under anaerobic conditions, disappearance of the absorption band of P_{fr} is accompanied by formation of an absorption band in the red. For leaves, the maximum is at 650 nm, for the internode sections at 665 nm. The difference spectra for this reaction are very similar to those for the phototransformation of the pigments and, therefore, mainly represent pigment reversal.

These results form a confirmation of our earlier conclusion that the photoreversible pigment systems in both organs are not completely identical.

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