REVERSIBLE CHANGES IN THE CIRCULAR DICHROISM OF PHYTOCHROME DURING PHOTOISOMERISATION OF THE PIGMENT

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Received May 10, 1968

Phytochrome is a chromophore-protein complex which acts as a light receptor for photoperiodic reactions in plants (Borthwick and Hendricks, 1960). The pigment complex is able to exist in two forms, which are reversibly interconvertible by irradiation with red (660 nm) or far red (720 nm) light:

$$P_R \underset{720\ nm}{\overset{660\ nm}{\rightleftharpoons}} P_{FR}$$

It is assumed that the photoisomerisation mechanism is based upon an isomerisation of the chromophore accompanied by a reversible change in the conformation of the protein part.

In several proteins, e.g. hemocyanin (van Holde, 1967), ferricytochrome c. (Zand and Vinogradov, 1967), ferredoxin (Palmer et al., 1967) and rhodopsin (Crescitelli et al., 1966, Takezaki and Kito, 1967), the prosthetic groups cause optical activity which shows up as circular dichroism in the region of their visible absorption bands. We have found that phytochrome displays a similar effect in the neighbourhood of its red and blue absorption bands. Moreover, interconversion of the two photoisomers causes concomitant changes in the circular dichroic effect, indicating that a conformational change is in fact involved.

MATERIALS AND METHODS

Phytochrome was isolated from 5-day old etiolated Avena seedlings by

two batch adsorptions on calcium phosphate gel, with an intermediate Sephadex G 50 gel filtration. The calcium phosphate gel was prepared according to the method of Tiselius et al. (1956). In this way, phytochrome was purified about 15 times from a specific activity of 0.002 $((OD_{660}-OD_{730})_{red}-(OD_{660}-OD_{730})_{far\,red})$ /cm/mg protein to 0.030 with a recovery of about 40%. The phytochrome preparations were then brought onto a DEAE-Sephadex G 50 column and eluted with a NaCl gradient. Fractions with phytochrome activity were pooled and the protein was precipitated by addition of an equal volume saturated $(NH_4)_2SO_4$ solution. The precipitate was dissolved in a minimal amount of buffer and gel filtrated over a Sephadex G 200 column. Specific activity and percentage recovery of phytochrome activity after each successive purification step are summarized in the Table.

Table

Purification of phytochrome extracted from etiolated oat seedlings

Stage of purification	Volume (ml)	Total act. Δ(ΔOD)/cm x volume (ml)	Spec. act. Δ(ΔOD)/cm/mg protein	Recovery of act. (%)
Raw extract	15,500	37.4	0.0016	100
Eluate from 2nd batch adsorption[1] on Ca-phosphate gel	57.5	15.2	0.0319	40.7
DEAE Sephadex G 50[2]; 1st peak fraction	60	4.4	0.1254	11.9
2nd peak fraction	105	2.9	0.0250	7.8
G 200 gel filtrate[3] of 1st peak fraction from DEAE	35.5	2.7	0.6900	7.1
$(NH_4)_2SO_4$ precipitate; of G 200 gel filtrate	2.5	1.7	0.7920	4.6

[1] Adsorption with 0.01 M K phosphate buffer, pH 6.2; desorption with 0.1 M K phosphate, pH 7.8.

[2] Column 40 x 2.5 cm; 0-1.0 M NaCl gradient in 0.01 M K phosphate buffer; pH 6.6.

[3] Column 50 x 2.0 cm; 0.01 M K phosphate buffer, pH 6.6.

Absorption spectra of the P_{FR} and P_R forms of phytochrome were measured in a Unicam SP 800 spectrophotometer in 1 cm cuvettes after irradiation of the solution with red and far red light respectively. For the activity calculations, optical densities at 660 and 730 nm were measured on a Zeiss PMQ II spectrophotometer. Red and far red light for irradiating samples was provided by a slide projector (Leitz Pradovit n 24, quartz-iodide lamp, 4250 Lumens) combined with interference filters (Schott, Mainz, type AL). Maximum transmittance of the filters was 57% at 660 nm and 52% at 720 nm and band width of the resultant light beams 20 nm.

Optical rotatory dispersion (ORD) of phytochrome solutions was measured with a Durrum-Jasco ORD recorder and circular dichroism (CD) with a Roussel-Jouan dichrograph.

RESULTS

Preliminary measurements of the ORD of a phytochrome solution with a specific activity of 0.250 showed that the 660 nm absorption band has a negative Cotton effect with a point of inflection at 664 nm (Fig. 1). Irradiation with red light for 4 minutes transforms the pigment into the P_{FR} form, causing a decrease in absorption at 660 nm accompanied by disappearance of the Cotton effect. The ORD curve of the original phytochrome solution showed another Cotton effect near the blue absorption band of P_R (max. 375 nm). This effect is superimposed upon the Drude curve for the protein and therefore not very clear.

Noise level of the Jasco recorder in the 600-700 nm region is high. Moreover, the P_{FR} absorption band is beyond the 700 nm limit of the instrument. Therefore, a second series of measurements was made with the Roussel-Jouan dichrograph of the CD up to 800 nm. In this case the phytochrome solution used was that with a specific activity of 0.792 (see Table and Fig. 2). A negative circular dichroic effect of P_R is found near the 660 nm band, while there is no optical activity in the 725 nm region. Irradiation with red light for 4 minutes forces the complex into a photostationary state with

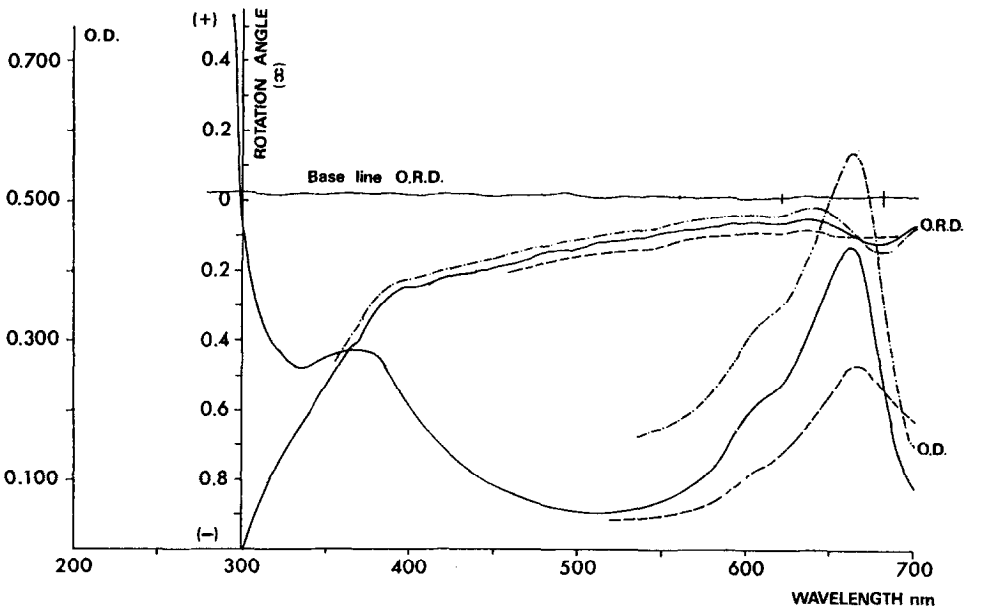

Fig. 1. ORD curves and absorption spectra of phytochrome
—— $P_{FR} + P_R$ (after 2 min far red irradiation)
---- P_{FR} (after 4 min red irradiation)
.-.- P_R (after 4 min far red irradiation)

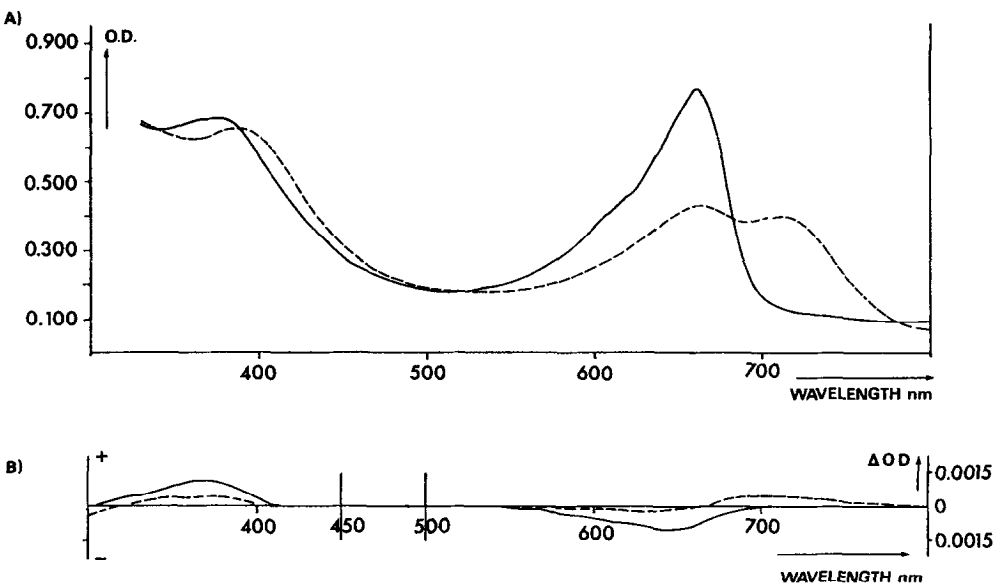

Fig. 2. CD curves and absorption spectra of phytochrome
A) Absorption spectra —— P_R ---- P_{FR}
B) Circular dichroism —— P_R ---- P_{FR}

$P_{FR}/P_R + P_{FR} = 0.8$ (Butler et al., 1964). The presence of non-transformed P_R is evident from the absorption spectra of P_{FR} in Figures 1 and 2. Thus the CD of P_{FR} displays a residual negative value at 660 nm, while the 725 nm absorption band of P_{FR} is associated with a positive circular dichroic effect.

Experiments starting with the P_{FR} form showed that the reverse reaction from P_{FR} to P_R under the influence of far red light is accompanied by a change in CD from a positive effect above 700 nm to a negative effect near 660 nm. No CD of the P_{FR} band remains after this transition because P_{FR} is completely converted to P_R.

The blue absorption band of phytochrome shows a red shift of about 15 nm (375 to 390 nm) when P_R is converted to P_{FR}. The blue absorption band of P_R exhibits positive CD, which diminishes upon irradiation with red light. The effect, however, does not change sign, but remains positive and finite.

DISCUSSION

The values of the observed circular dichroic effects are small ($\Delta OD_{max} = \sim 0.001$ for an absorbance of 0.760 at 660 nm). If we assume that the phytochrome chromophore has a biliverdinoid structure (Siegelman et al., 1966), it is likely that the optical activity of the chromophore is induced by its association with the protein in the pigment complex (Moscowitz et al., 1964). The intensity of the red and far red absorption bands of phytochrome decreases strongly upon denaturation (Butler et al., 1964b) which indicates that energy transfer is possible between chromophore and protein. The existence of a weak charge-transfer complex between the phytochrome chromophore and amino acid side chains of the protein moiety therefore seems possible (Carrion et al., 1967). The reversible photoisomerisation mechanism of phytochrome could then be explained by the uptake of an amount of light energy which causes an isomerisation of the chromophore and leads to a transition state. This extra energy induces a local conformational change in the protein and results in the formation of another charge-transfer complex by interaction with other amino acid side chains of the protein. The weak optical effect of

P_R and P_{FR} in this context then becomes clear if it is supposed that a proton shift or perturbation in the chromophore is stabilized when the protein part adopts either of two particular conformational states.

Another protein pigment which has properties in common with phytochrome is C-phycocyanin (Siegelman et al., 1966). Boucher et al. (1966) measured its ORD. The red and the blue absorption bands of this pigment also have optical activity but, in both cases, rotation is in the opposite sense to that of the corresponding phytochrome bands. We measured the circular dichroism of both C-phycocyanin and its free chromophore and found that optical activity of the latter was very low.

Only indirect information about conformational changes in the protein part of phytochrome can be obtained from the experiments described in this article. These changes are comparable with the allosteric transitions in hemoglobin and certain enzymes and not with the loss of conformation upon denaturation of phytochrome described by Butler et al., 1964b. Although one would not expect such minor changes in protein conformation to show up as large effects in the UV region of the spectrum, we will nevertheless try to measure the CD of phytochrome in the far ultraviolet range.

ACKNOWLEDGEMENTS

The author's sincere thanks are due to Mr. E.H.M. Greuell, U.R.L. Duiven and Prof. Dr. R. Schwyzer, Laboratory of Molecular Biology E.T.H. Zürich, for helpful discussions. The latter made the ORD recorder available, while Dr. C.A. Emeis of the Theoretical Organic Chemistry Department of Leyden University allowed me to use their dichrograph. Phytochrome preparations were isolated and purified with the expert assistance of Miss J.M. Geers and Mr. A. van Rooijen.

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