that the lipid was in fact completely reduced under the experimental conditions. When the solutions were chromatographed, glucosamine and glucosaminol moved several cm in the solvent chosen, while chitobiose and the reduced fraction remained at the origin, indicating that the reduced fraction does not contain single units of amino sugar. This stable disaccharide must be isolated and characterized to confirm these observations. Other questions about the reduction experiments were raised by side fractions, one accounting for about 5% of the nitrogen originally present in the lipid, in which the nitrogen-phosphorus ratio is considerably higher than 2, and one accounting for 10% of the lipid nitrogen, in which the same ratio is less than 2. We feel that these fractions represent some degradation of the two-unit structure under the conditions used to reduce the lipid and to remove excess sodium borohydride following the reaction.

The results of the sodium borohydride reduction and alkaline degradation indicate, however, that the two glucosamine molecules are linked glycosidically; after complete reduction a fraction is obtained in which approximately half the amino sugar present is still glucosamine. The apparent stability of the reduced unit to vigorous alkaline hydrolysis indicates that the glycoside joins two glucosamine molecules without involving an hydroxy fatty acid: either an amide or an ester of the latter with a second glucosamine molecule should cleave under the conditions of vigorous alkaline hydrolysis. A phosphodiester linkage does not seem likely; two glucosamine molecules so joined should be completely reduced to glucosaminol. Such a linkage should also cleave under vigorous alkaline conditions. A unit in which a glycosidic bond joins two molecules of glucosamine is therefore tentatively proposed as the basic structural unit of Lipid A. Confirmation of this structure will depend on the outcome of similar experiments conducted on a larger scale.

In conclusion it seems worthwhile to consider how Lipid A is attached to the lipopolysaccharide. Several linkages are possible, including again the glycosidic and phosphodiester bonds, as well as that provided by a di-functional amino acid. A glycosidic bond in which the Lipid A moiety represents a terminal unit attached to a polysaccharide chain is an attractive hypothesis. Studies on the borohydride reduction of intact lipopolysaccharide might well provide decisive evidence on this point.

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Purification of Phytochrome from Oat Seedlings

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Phytochrome, the photoreceptor controlling many aspects of growth and development of higher plants, was extracted from dark-grown Clintland oats. A 60-fold purification was achieved by methods of protein separation: ultrafiltration, calcium phosphate chromatography, gel filtration on Sephadex G-200, and DEAE-cellulose chromatography. The chromoprotein has a molecular weight of between 90,000 and 150,000 and the purity of highest specific activity fractions is estimated to exceed 30%.

Many aspects of plant growth and development are controlled by light quality and the daily duration of light and darkness. Action spectra for the effects of light on flowering, etiolation, germination of light-responsive seeds, plastid development, and many other plant responses (Borthwick and Hendricks, 1960) indicate uniquely that the same reversible photochromic pigment is the effective photoreceptor in all these

responses. The pigment, now called phytochrome (P),[1] was found to have two distinct forms (Pr and Pfr) with action maxima near 660 and 730 mμ. These two forms were interconverted by the action of red and far-red light as follows:

$$Pr \underset{730\ m\mu}{\overset{660\ m\mu}{\rightleftharpoons}} Pfr$$

Pfr is presumed to be an enzyme or coenzyme which controls a reaction common to a number of metabolic pathways.

Phytochrome was detected photometrically *in vivo* by measuring the reversible spectral changes following irradiation with red and far-red light (Butler *et al.*, 1959). The photometric assay detected phytochrome extracted from plants, and purification was achieved by using methods of protein separation. A convenient and reproducible purification of phytochrome, a blue chromoprotein, from dark-grown oat seedlings is described here.

Experimental Procedure

Lots of about 650 g dry oat seed, *Avena sativa* L. cv Clintland, obtained from Southern States Cooperative, Inc., Baltimore, Md., were evenly distributed on each of seven 18 × 26–in. aluminum baking pans lined with two sheets of 0.25-in. cellulose wadding, type 2011 (Kimberley-Clark Corp., Neenah, Wisc.), moistened with 2 liters of tap water. The pans were placed in a light-proof 71.5 × 21.5 × 28–in. aluminum storage cabinet (Bucks County Enterprises, Inc., Quakertown, Pa.), covered with black sateen cloth, and held in a dark incubator at 24°. After 5 days the cabinet was transferred to a 2° cold room without exposure of the seedlings to light. At this stage the seedlings were about 8 cm tall. The pans of seedlings, cooled overnight, were removed from the cabinet, and the seedlings were harvested by cutting with scissors below the coleoptilar node, avoiding the inclusion of seed.

All operations of extraction and purification were performed under incandescent illumination in a cold room (2°). A schematic diagram of the fractionation procedure is shown in Figure 1.

Extraction.—In a typical preparation 1.5 kg of harvested tissue was placed in a stainless-steel bucket with 1.5 liters of an extraction solution consisting of 0.1 M sodium pyrophosphate, 0.07 M 2-mercaptoethanol, 0.002 M EDTA, and 75 g of Solka-Floc SW-40-B cellulose (Brown Co., Berlin, N. H.). The tissue with solution was ground in a stainless-steel food chopper (Hobart Mfg., Co., Troy, Ohio), which had a cutting plate with 7-mm openings. The resultant slurry was filtered on two 240-cm Büchner funnels through a compacted 1/4-in. bed of cellulose, using a rubber dam to aid the vacuum filtration. The filtrate was centrifuged for 25 minutes at 16,000 × *g*. The volume of supernatant was about 2.5 liters.

Ultrafiltration and Gel Filtration.—The supernatant solution from the above step was concentrated approximately 4-fold by ultrafiltration (Siegelman and Firer, 1962) in 18 hours and then centrifuged for 20 minutes at 37,000 × *g*. The total supernatant was loaded on a 10 × 60–cm column of Sephadex G-50 medium grade (Pharmacia Fine Chemicals, Rochester, Minn.)

[1] Abbreviations used in this work: P, phytochrome; Pr, phytochrome interconverted by the action of red light; Pfr, phytochrome interconverted by the action of far-red light; DEAE-, diethylaminoethyl-; EDTA, ethylenediaminetetraacetate.

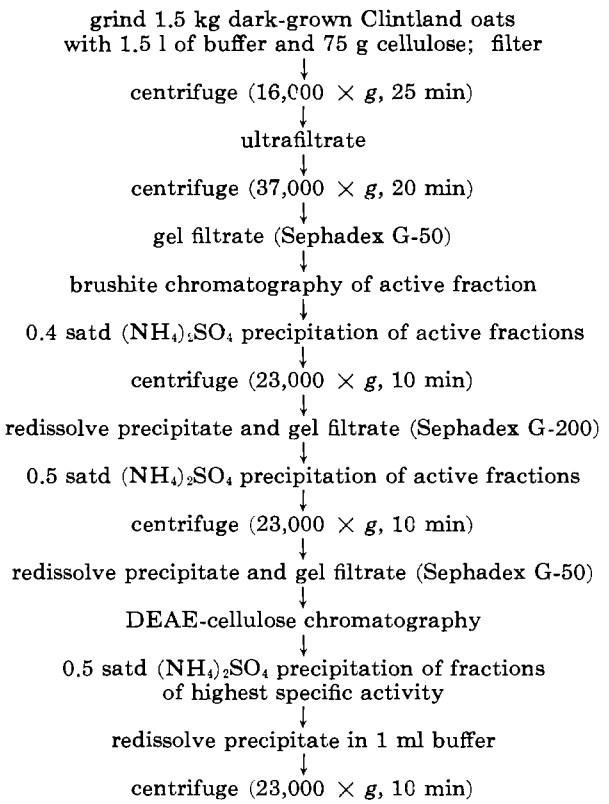

Fig. 1.—Schematic procedure for purification of phytochrome from dark-grown Clintland oats. See text for details.

equilibrated with 0.01 M potassium phosphate buffer, *p*H 7.8. All buffer solutions used throughout the following procedures contained 0.05 M 2-mercaptoethanol and 0.001 M EDTA. The column was operated with an 8-ft hydrostatic head and had a flow rate of about 6 ml/minute. The column was eluted with 0.01 M potassium phosphate buffer, *p*H 7.8. The phytochrome-containing fraction can be monitored visually because it accompanies the first yellow band moving down the column.

Calcium Phosphate Chromatography.—A 7 × 25–cm column of partially altered brushite was equilibrated with 1 liter of 0.01 M potassium phosphate buffer, *p*H 7.8. The phytochrome-containing solution from the previous step was loaded on the column with about a 5-ft hydrostatic head. Channeling, due to shrinkage of the brushite on adsorption of the protein, was eliminated by thoroughly stirring the bed to about 2 cm below the loaded portion of the column if necessary. The column was washed with 1.5 liters of equilibration buffer. A slurry of brushite was poured on top of the column to form a protective layer about 1.5 cm thick. A disk of porous linear polyethylene (Porex Materials Corp., Fairburn, Ga.) 0.10 in. thick was floated on the buffer above the column bed. The height of the column above the fraction collector was adjusted until a flow rate of 2 ml/minute was obtained. The excess buffer above the top of the column was removed until the polyethylene disk was about 1 mm above the column bed. The column was eluted by the use of an exponential gradient mixer containing a starting buffer of 150 ml of 0.01 M potassium phosphate, *p*H 7.8, and a limit buffer of 1500 ml of 0.4 M potassium phosphate, *p*H 7.8. The flow rate (120 ml/hour) was kept constant by use of a peristaltic pump (Sigmamotor Inc., Middleport, N. Y.). The fractions containing phytochrome were greenish yellow and could be visually detected. The combined active fractions were pre-

cipitated by rapid addition of 0.7 vol of saturated ammonium sulfate solution previously adjusted to *p*H 7.5 with ammonium hydroxide. The suspension was stirred for 10 minutes and then centrifuged for 10 minutes at 23,000 × *g*. The supernatant was discarded and a light-green precipitate was obtained.

Sephadex G-200 Gel Filtration.—The light-green precipitate from the previous step was well dispersed in 6 ml of 0.01 M potassium phosphate buffer, *p*H 7.8 and centrifuged for 10 minutes at 23,000 × *g*, and the green supernatant solution was poured off. The remaining precipitate was well dispersed in 3 ml of 0.01 M potassium phosphate buffer, *p*H 7.8, and centrifuged for 10 minutes at 37,000 × *g*. An additional extraction of the ammonium sulfate precipitate may be required if a jellylike precipitate is obtained. The supernatants were combined and had a total volume of 15 ml. The combined supernatants were applied to a 7 × 40–cm column of Sephadex G-200 equilibrated with 0.25 M potassium phosphate buffer, *p*H 7.8. Loading the sample on the column was aided with a disk of porous polyethylene floated in the buffer above the column bed. The buffer above the bed was removed until the polyethylene disk rested on top of the bed and a meniscus was seen just at the junction of the polyethylene disk and the column. The combined supernatant solution was pipetted on the polyethylene disk. The colored solution was allowed to sink into the column and then the equilibration buffer was pipetted on the polyethylene disk. The disk protected the sample from mixing and dilution. The column was operated with about an 85-cm hydrostatic head. Flow rate from the column was kept constant at 60 ml/hour by use of a pump on the outlet of the column. The fractions of highest activity were combined and an equal volume of saturated ammonium sulfate was added rapidly. The suspension was stirred for 10 minutes and centrifuged for 10 minutes at 23,000 × *g*, yielding a blue precipitate. The blue precipitate was well dispersed with 6 ml of 0.01 M potassium phosphate buffer, *p*H 7.8. The mixture was centrifuged for 10 minutes at 23,000 × *g*, yielding a clear blue-green solution.

DEAE-cellulose Chromatography.—A 2.5 × 55–cm column of DEAE-cellulose (0.3 meq exchange capacity) was equilibrated with 500 ml of 0.01 M potassium phosphate buffer, *p*H 7.8, containing 0.001 M EDTA, but 2-mercaptoethanol was omitted throughout this step. The blue-green solution from the previous step was equilibrated with column buffer by passage through a 2 × 24–cm column of Sephadex G-50. It was then applied to the DEAE-cellulose column and 200 ml of the equilibration buffer was passed through the column. The column was eluted, using a linear gradient mixer containing a starting buffer of 800 ml of 0.01 M potassium phosphate, *p*H 7.8, and limit buffer of an equal weight of 0.25 M potassium phosphate buffer, *p*H 7.8. The flow rate (60 ml/hour) was kept constant with a pump and 5-ml fractions were collected. The fractions were assayed for phytochrome absorbancy changes and 280-mμ absorbance. Fractions with highest specific activity were combined and precipitated by the rapid addition of an equal volume of saturated ammonium sulfate solution. The suspension was stirred for 15 minutes and then centrifuged for 10 minutes at 23,000 × *g*. The supernatant was discarded and the blue-green precipitate was taken up in 1 ml of 0.01 M potassium phosphate buffer, *p*H 7.8. The solution obtained was centrifuged for 10 minutes at 23,000 × *g* and the clear blue-green supernatant solution was examined.

Phytochrome Assay.—The pigment was measured with a dual–monochromator spectrophotometer (Birth and Norris, 1963). A similar commercial instrument is now available (Agricultural Specialities Co., Hyattsville, Md.). The assay for phytochrome was made by measuring the optical-density difference in a 1- or 5-cm path-length cell between 660 and 730 mμ after irradiation with red and far-red light. The red-irradiation source was obtained with a 650-mμ interference filter and the far-red-irradiation source with a red cutoff filter (Corning No. 2030) and four layers of dark-blue cellophane. A tungsten-filament lamp was used as the light source. The optical-density difference between 660 and 730 mμ ($\Delta OD = OD_{660} - OD_{730}$) was measured after first red and then far-red radiation. The difference between these two readings [$\Delta(\Delta OD)$] is a measure of the total amount of reversible phytochrome.

Protein Determination.—Protein was estimated by the biuret procedure (Gornall *et al.*, 1949) after trichloroacetic acid precipitation and washing with ethanol. After the Sephadex G-200 gel-filtration step and removal of mercaptoethanol, biuret determinations and 280 mμ absorbance were well correlated and the latter was used for protein estimation.

Absorbance Measurements.—Absorbance at 280 mμ of column eluates was measured with a Beckman DU spectrophotometer. The absorption spectrum of phytochrome was determined with a Cary Model 14 spectrophotometer in a 1-cm cell at 2° in 0.01 M potassium phosphate buffer, *p*H 7.8.

Calcium Phosphate.—Granular altered brushite, $CaHPO_4 \cdot 2H_2O$ of excellent flow characteristics was prepared by a combination of procedures (Jenkins, 1962; Neurnan *et al.*, 1962). Fifteen liters each of 0.5 M solutions of calcium chloride and potassium monohydrogen phosphate were pumped at about 10 ml/minute into a 50-liter Pyrex jar and vigorously stirred. The resulting precipitate of brushite was held at room temperature for about 7 days and then washed five times by decantation with distilled water to remove fines and salts. The washed solid was stored at 2° in distilled water. Columns were prepared by pouring a thick slurry of the brushite into the column at room temperature.

Sephadex.—Sephadex G-50 was prepared for use by allowing the material to swell in a large volume of 0.05 M sodium chloride overnight. The fine particles were removed by decantation in 0.05 M sodium chloride, allowing the coarse particles 10 minutes to settle. This procedure was done 15 times. Dry Sephadex G-200 (bed volume per gram of dry gel, 15–20 ml) was sieved and the particles 200 mesh or larger in size only were allowed to swell in 0.25 M potassium phosphate buffer, *p*H 7.8, for 48 hours. Minimal fines were removed by decantation. The Sephadex columns were formed by pouring a thick slurry of the bed material in the column, and a porous polyethylene disk was placed on top of the column.

Diethylaminoethyl-cellulose.—The DEAE-cellulose anion exchanger (0.3 meq exchange capacity per gram) was made with SW-40-B Solka-Floc cellulose (Peterson and Sober, 1961). The ion exchanger was regenerated by washing on a large sintered-glass funnel with water, ethanol, 0.5 M NaOH, and water until neutral to phenolphthalein. The cake was well dispersed in water and adjusted to *p*H 7.8 at the glass electrode with minimal additions of molar solutions of potassium mono- and dihydrogen phosphate while vigorously stirring. The DEAE-cellulose columns were packed at 6 pounds pressure.

Analytical electrophoretic examination was performed by disc electrophoresis (Davis and Ornstein,

TABLE I
SUMMARY OF PURIFICATION OF PHYTOCHROME FROM CLINTLAND OATS

Fraction	Volume (ml)	Activity (Δ(ΔOD)/cm)	Total Activity (Δ(ΔOD)/cm²)	Total Protein (mg)	Specific Activity	Recovery (%)	Purification
(1) Initial extract	2500	0 0038	9.5	4630	0.0021	100	1.0
(2) Ultrafiltrate	590	0.0126	7.4	2480	0.0030	78	1.4
(3) Sephadex G-50 eluate	695	0.0108	7.5	2010	0.0037	79	1.8
(4) Calcium phosphate eluate	166	0.0325	5.4	980	0.0055	57	2.6
(5) Sephadex G-200 eluate and ammonium sulfate precipitation	6	0.64	3.85	223	0.017	41	8.1
(6) DEAE-cellulose eluate and ammonium sulfate precipitation (highest specific activity fraction)	1	0.58	0.58	4.5	0.129	6.2	61

1959), omitting the upper gel. The protein solution in 0.2 M sucrose was pipetted directly on top of the 7.5% acrylamide gel. Electrophoresis was carried out at a constant current of 2.0 ma/gel column for 4 hours at 5°. A Spinco Analytrol densitometer was used to scan the amido-black stained gels. Phytochrome was detected directly on the gel before the protein was stained.

The sedimentation coefficient was determined by the band centrifugation procedure (Vinograd *et al.*, 1963), and molecular weight was estimated by equilibrium sedimentation (Yphantis, 1962).

Remarks.—There are several requirements for successfully extracting and purifying phytochrome from plant tissue. Only seedlings grown in absolute darkness at a favorable temperature for growth contain the maximal content of the pigment. The pigment can be extracted in many ways. High-speed homogenizers were found to be least satisfactory. The most exhaustive extraction was obtained by milling the seedlings at −78°. However, undesirable materials such as polysaccharides and a nontransformable protochlorophyll, which interfered with purification, were also solubilized. The most satisfactory extractions were made with either the mortar and pestle or a large food chopper. Although these two grinding procedures did not extract the maximal amount of pigment from the tissue, they provided initial extracts which were readily purified. The amount of phytochrome obtained in solution also depends on the final *p*H after the initial extraction. At *p*H 7.3 or above the supernatant solution after filtration and centrifugation contained the pigment. At *p*H 6.2 or below the phytochrome was in the sediment. These results were independent of the buffer used.

RESULTS

A rapid and reproducible procedure for the extraction and purification of phytochrome was achieved with dark-grown Clintland oat seedlings as a source of the chromoprotein. A typical purification of 2.5 liters of an initial extract is summarized in Table I. Ultrafiltration and Sephadex G-50 gel filtration removed much colored material, concentrated the solution for convenience of handling, and reduced the salt concentration for the following adsorption step. An elution diagram of 280 mμ absorbance and phytochrome absorbancy changes from the calcium phosphate column is shown in Figure 2. There was a separation of phytochrome from other proteins but no indication of a fractionation is seen from 280 mμ absorbance. Much yellow-colored material is not eluted from the column even with 1.0 M K_2HPO_4. Phytochrome can be observed as a green band moving down the column. The photoreversible absorbance of the pigment can be visually detected at this stage by irradiating the green band on the column with a red or far-red source and observing the diminution or enhancement of the color. The calcium phosphate eluate was concentrated and applied to a column of Sephadex G-200. An elution diagram of 280 mμ absorbance and phytochrome absorbancy changes from the Sephadex G-200 column is shown in Figure 3.

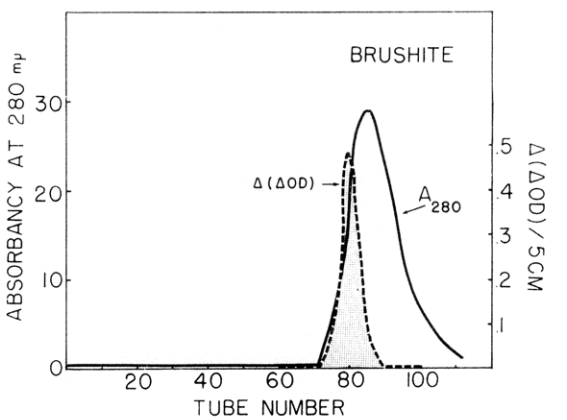

FIG. 2.—Chromatography of oat phytochrome on a 7 × 24-cm column of calcium phosphate. The column was initially washed with 1.5 liters of 0.01 M potassium phosphate buffer, *p*H 7.8, and then eluted with gradient to 0.5 M potassium phosphate buffer, *p*H 7.8, at 2 ml/minute. The effluent was collected in 10-ml fractions. See text for details.

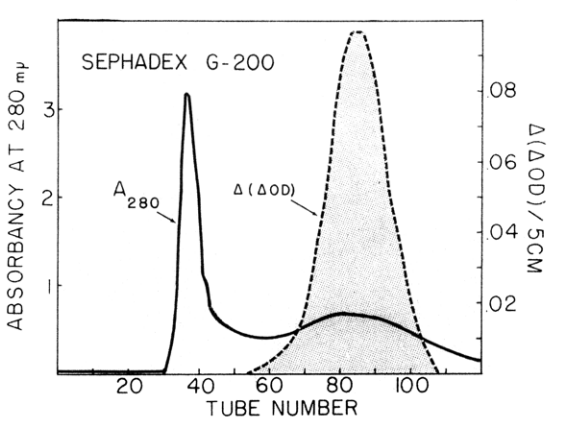

FIG. 3.—Gel filtration of oat phytochrome on a 7 × 36-cm column of Sephadex G-200 with 0.25 M potassium phosphate buffer, *p*H 7.8, as eluent. Flow rate was 1 ml/minute, and the effluent was collected in 10-ml fractions. See text for details.

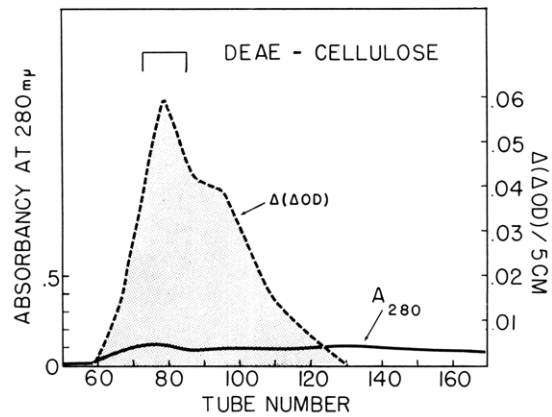

FIG. 4.—Chromatography of oat phytochrome on a 2.5 × 55-cm column of DEAE-cellulose. The column was initially washed with 200 ml of 0.01 M potassium phosphate buffer, *p*H 7.8, and then eluted with a gradient to 0.25 M potassium phosphate buffer, *p*H 7.8, at 1 ml/minute. The effluent was collected in 5-ml fractions. The highest specific activity fractions, indicated by the bracket, were combined. See text for details.

A prominent yellow band was observed to move faster than phytochrome on this column. On separation of the yellow band the phytochrome became bluer and the photoreversibility was easily detected on the column. The active fractions from the Sephadex G-200 gel filtration were concentrated and applied to a column of DEAE-cellulose. An elution diagram of 280 mμ absorbance and phytochrome absorbancy changes is shown in Figure 4. There is little obvious parallel of 280 mμ absorbance and phytochrome absorbancy changes. The tubes under the bracket in Figure 4 were combined and concentrated. The absorbance of these combined tubes is shown in Figure 5.

The highest specific activity fraction from the DEAE-cellulose chromatography was examined by disc electrophoresis and analytical centrifugation. Disc electrophoresis resolved the fraction into a leading blue band and a trailing yellow-green band. The blue band showed the photoreversible absorbance changes after red and far-red irradiation. The location of phytochrome on the gel following electrophoresis and the protein bands after staining with amido black are shown in Figure 6. Analytical-centrifuge studies of the highest specific activity fraction indicated a sedimentation coefficient, $s_{20,w}$ of 4.5 and a weight-average molecular weight of between 90,000 and

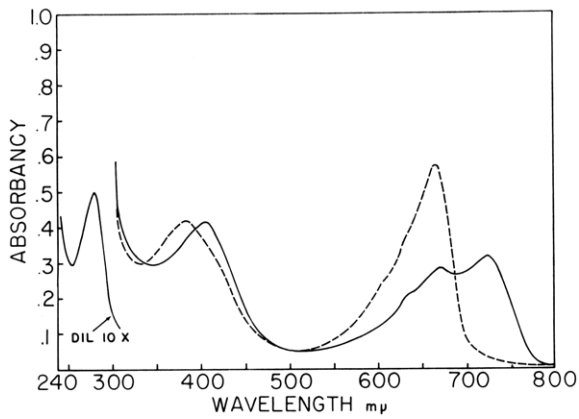

FIG. 5.—Absorbance of the combined highest specific activity fractions of oat phytochrome after DEAE-cellulose chromatography. After red irradiation (solid line), and after far-red irradiation (dashed line).

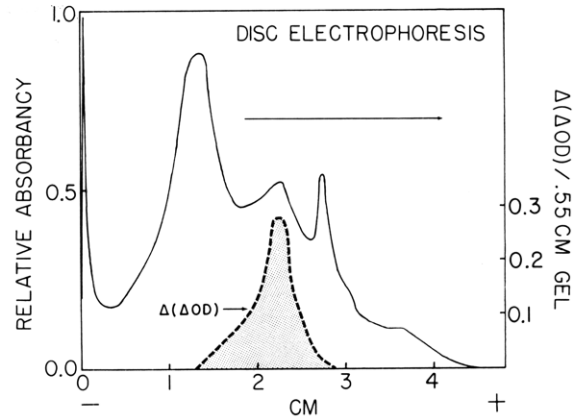

FIG. 6.—Disc electrophoresis of the combined highest specific activity fractions of oat phytochrome after DEAE-cellulose chromatography. Densitometer tracing of amido-black-stained protein (solid line). Phytochrome absorbancy changes assayed directly on the acrylamide gel (dashed line).

150,000. The heterogeneity of the preparation did not permit a more precise estimate of molecular weight.

DISCUSSION

The purification of phytochrome required a suitable method of detection since no biochemical or biological assay was available. A differential spectrophotometric assay was developed which measured the photoreversible changes of the pigment. However, the sensitivity of the measurement, while high for spectrophotometry is low compared with that of usual enzymatic assays.

Several plant materials were compared spectroscopically *in vivo* as a possible source of phytochrome Dark-grown seedlings were found to have more pigment than other plant parts examined. Oat seedlings were finally selected as favorable material for isolation of the chromoprotein. They contained more than twice as much pigment per gram fresh weight of the shoot as was found in other plants examined. Uniform seed is commercially available and the seedlings are easily grown. Phytochrome from oat seedlings is sufficiently stable at 2° for 2 weeks to permit its purification. Storage at −15° in 0.01 M potassium phosphate buffer, *p*H 7.8, was not successful. Initially, corn seedlings were used as a source of the chromoprotein. The lower amount of pigment per gram fresh weight, the difficulty of separation of yellow contaminants, and the need for an initial DEAE-cellulose adsorption to remove most brown-colored contaminants precluded use of corn. Barley seedlings were next tried as a source of the pigment. The phytochrome separated from barley was found to be less stable than that obtained from corn.

Phytochrome is a chromoprotein. It was purified by methods of protein chemistry, and the biuret test to assay protein was used. Prominent ultraviolet absorbancy of plant phenolic compounds precludes the use of 280 mμ absorbance as a measure of protein concentration in plant extracts. The photoreversible absorbance is destroyed by *p*H values below 4 and above 10, at 60°, 4 M urea, freezing and thawing, and trypsin digestion. The unique spectral properties are the result of a specific chromophoric group conjugated with a native protein. The chromophore is firmly attached to the protein and is not removed by gel filtration, prolonged dialysis, or trichloroacetic acid precipitation. No attempts have yet been made

to cleave the chromophore from the protein. Reducing agents such as 0.05 M mercaptoethanol and metal-complexing agents such as 0.01 M EDTA have no effect on spectral behavior. No evidence for a dissociable factor or the need for an external oxidant or reductant for photoreversibility was found. The spectral changes probably arise from a molecular rearrangement of the chromophore.

The long-wavelength maxima of phytochrome from action spectra, *in-vivo*, and *in-vitro* spectrophotometric measurements are near 670 and 725 mμ. The ratio of the photoreversible absorbancies of the two forms of the pigment is about 1:1 *in vivo* and *in vitro* from preparations of corn and oats. This ratio was 2:1 for soluble barley preparations, and insoluble preparations had ratios as high as 5:1. The inverse relation between increased absorbancy ratios and decreased solubility indicated that denaturation can be measured by the absorbancy ratio between the long-wavelength maxima. Absorbance spectra of phytochrome gave the first clear evidence of other photoreversible absorption bands in the blue and ultraviolet portions of the spectrum. No thermal transformation of phytochrome separated from oats *in vitro* was observed on holding 3 hours at room temperature in the Pr or the Pfr form.

The intracellular location of phytochrome is not known. The marked influence of *p*H on the solubility of the chromoprotein near neutrality makes any decision on the location of the pigment tenuous (Gordon, 1961).

The structure of the chromophore of phytochrome is presently unknown. Comparison of the absorbance spectra of allophycocyanin (Ó hEocha, 1962), a biliprotein, and phycobilin (Ó hEocha, 1963) suggests that the chromophore of phytochrome may be a bile pigment.

The purification of the highest specific activity phytochrome in these studies is about 60-fold. From considered estimates of contamination by other proteins from disc electrophoresis and analytical centrifugation, the purity of the highest specific activity fraction exceeds 30%. Therefore, although the purification is not high, the purity is noteworthy, and attempts to achieve further purification are under way. Using a probable molar absorbancy of 2×10^4 (Butler, 1961), the number of chromophores per molecule is likely to be one and would probably not exceed two.

Naturally occurring photoreceptors such as the photosynthetic and visual pigments are relatively few in number. Phytochrome purification has established that the pigment is a chromoprotein whose spectral behavior closely parallels the action spectra for the photoresponsive control of plant growth and development. Obtaining phytochrome in purified form is important for understanding the biochemical control of growth in higher plants. The purity and amounts of chromoprotein which can be obtained by the procedures described herein are adequate for examination of the chemical nature of the chromophore, for photochemical studies of the photoreversible behavior of the pigment (Hendricks *et al.*, 1962), and for comparison of the pigment isolated from several plant sources.

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Mention of commercial names does not imply indorsement by the U. S. Department of Agriculture.

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