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Immunopurification and Initial Characterization of Dicotyledonous Phytochrome[1, 2]

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MARIE-MICHÈLE CORDONNIER[3] AND LEE H. PRATT
Botany Department, University of Georgia, Athens, Georgia 30602

ABSTRACT

Antiserum was prepared against proteolytically undegraded phytochrome obtained from etiolated zucchini squash (*Cucurbita pepo* L., cv. Black Beauty). The antiserum was prepared by injecting into a rabbit immunoprecipitates between zucchini phytochrome and specific antiserum against undegraded oat (*Avena sativa* L., cv. Garry) phytochrome. Specific antiphytochrome immunoglobulins were purified from this crude serum by an affinity column consisting of conventionally purified undegraded pea phytochrome covalently linked to cyanogen bromide-activated agarose. These purified immunoglobulins were also linked to cyanogen bromide-activated agarose and were used to immunopurify zucchini, pea (*Pisum sativum* L., cv. Alaska), and lettuce (*Lactuca sativa* L., cv. Grand Rapids) phytochrome. All three dicotyledonous phytochromes exhibited a monomer size near 120,000 daltons by sodium dodecyl sulfate, polyacrylamide gel electrophoresis. Absorbance spectra of immunopurified zucchini phytochrome indicated that the ratio of visible to ultraviolet absorbance for purified zucchini phytochrome is lower than that observed for oat phytochrome. The isoelectric point of zucchini phytochrome, which was observed to be heterogeneous by this criterion, was found to be in the range of 6.5 to 7.0, higher than that observed for oat phytochrome. The electrophoretic mobility of zucchini phytochrome was found to be similar to that observed for oat and pea phytochrome under conditions that were nondenaturing and did not involve any molecular sieving effect. The amino acid analysis of zucchini phytochrome is similar to that reported previously for oat and rye (*Secale cereale* L., cv. Balbo) phytochrome.

The biochemical and biophysical properties and intracellular distribution of dicotyledonous phytochrome have been poorly investigated by comparison to monocotyledonous phytochrome (13 and 25 for reviews). If one assumes, however, that phytochrome functions in the same way in all plants, then it is important to initiate more intensive biochemical and biophysical studies of dicotyledonous phytochrome. Comparative investigations of both monocotyledonous and dicotyledonous phytochrome should reveal those properties that are in common and are thus most likely related to its mode of action.

To obtain maximum information about dicotyledonous phytochrome, it will be necessary to utilize both spectrophotometric and immunochemical assays (14 for discussion). Because antimonocotyledonous-phytochrome serum recognizes dicotyledonous phytochrome poorly (4, 12, 21), it is necessary to develop independently antiserum against dicotyledonous phytochrome. This antiserum could then be used for several purposes, including immunopurification of dicotyledonous phytochrome (8), its quantitation by radioimmunoassay (15), and determination of its intracellular distribution by immunocytochemistry (15).

Although what little *in vitro* work that has been done with undegraded dicotyledonous phytochrome has been done almost exclusively with pea phytochrome (4, 12, 21, 28, 29), it seemed of most interest to begin a study of dicotyledonous phytochrome with that isolated from zucchini squash for two reasons. First, zucchini phytochrome has been studied extensively with regard to light-induced phytochrome pelletability (13 and 18 for reviews), a phenomenon that might be related to the binding of phytochrome with a membrane receptor (10). Inasmuch as the results of these investigations have been controversial (13 and 18 for discussions) more knowledge about zucchini phytochrome should assist in the interpretation of these phytochrome pelletability studies. Second, zucchini phytochrome has been used already for some *in vitro* investigations (19, 20). Development of an immunochemical assay capability for zucchini phytochrome should permit extension of these initial observations.

The goals of the work reported here are: (a) to develop an antiserum against zucchini phytochrome, (b) to utilize this antiserum for the immunopurification of dicotyledonous phytochrome, and (c) to initiate the characterization of a dicotyledonous phytochrome.

MATERIALS AND METHODS

Zucchini (*Cucurbita pepo* L., cv. Black Beauty), peas (*Pisum sativum* L., cv. Alaska), oats (*Avena sativa* L., cv. Garry) and lettuce (*Lactuca sativa* L., cv. Grand Rapids) were grown in darkness at 25°C and near saturating humidity on open, plastic cafeteria trays covered with cellulose packing material (Kimpak 6234, 62360, or 64290, Kimberly-Clark, Neenah, WI). Plants were harvested after 5 (lettuce, zucchini, and oats) or 7 (peas) days. Lettuce seedlings were grown with a sheet of Miracloth (Calbiochem, La Jolla, CA) over the Kimpak and they were harvested intact by scraping them from the Miracloth. Zucchini shoots were harvested after removing the cotyledons. For most extractions, only the hook region was used. Oats and peas were harvested as before (12). Harvested tissue was stored at −20°C until extracted.

All experimental work was performed under controlled green illumination (12, 16) until immunoprecipitates were collected or phytochrome was denatured. Plant extracts were kept between 0 and 4°C. Phytochrome preparations were stored at or below −70°C.

Conventional Phytochrome Preparations. Pea phytochrome that

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[3] Present address: Laboratoire de Physiologie Végétale, Université de Genève, 3 place de l'Université, 1211 Geneva 4, Switzerland.

had a SAR[4] of 0.18 and that was used for the purification of antiphytochrome immunoglobulins and for immunoelectrophoresis (Figs. 3 and 8) was purified by brushite chromatography, ammonium sulfate fractionation, and DEAE-cellulose and Bio-Gel P-300 (Bio-Rad, Richmond, CA) chromatography as described earlier (12). This phytochrome preparation was stored in 70 mM K-phosphate (pH 7.8). Other phytochrome preparations were partially purified by brushite chromatography and 20 g/100 ml ammonium sulfate fractionation ("brushite purified") (12). For lettuce phytochrome preparations, the crude extract and all subsequent buffers used during purification were 2 mM in phenylmethylsulfonyl fluoride, 2 mM in benzamidine, and 10 mM in ϵ-amino-*n*-caproic acid to minimize protease activity. These other phytochrome preparations were stored in 0.1 M Na-phosphate (pH 7.8).

One unit of phytochrome is defined here as that quantity which in 1.0 ml gives an $A_{667}^{1\,cm} = 1.0$ after a saturating far-red irradiation. Phytochrome purity is estimated by its SAR and/or by SDS-polyacrylamide gel electrophoresis (14).

Immunopurification of Phytochrome. Dicotyledonous phytochrome was immunopurified as described by Hunt and Pratt (8). The following minor modifications were incorporated into the protocol. Agarose-immobilized antizucchini-phytochrome immunoglobulins (see below) were kept throughout in a syringe, which was operated as a small chromatographic column. Crude or brushite-purified phytochrome solutions were incubated with the immobilized immunoglobulins for about 30 min either by mixing with the immobilized immunoglobulins in the syringe or more often by passing the solutions slowly through the syringe over a period of about 15 min. Adsorbed phytochrome solutions were saved and adsorbed repeatedly after each 3 M $MgCl_2$ elution, omitting the intervening 1 N HCOOH elution (8), as long as the concentration of phytochrome in the adsorbed preparation was greater than 0.05 unit/ml. After the final $MgCl_2$ elution, a final HCOOH elution was performed and the beads were washed and prepared for subsequent use as described elsewhere (8). When phytochrome was being eluted from the immunocolumn, the eluate of this column was passed immediately through a Sephadex G-25 (Pharmacia, Piscataway, NJ) column to separate phytochrome from the eluting agent. All buffers and the 3 M $MgCl_2$ solution were at pH 7.8 rather than the previously used 7.5. Additional details concerning this immunopurification procedure are available elsewhere (16).

Gel Electrophoresis. SDS-polyacrylamide gel electrophoresis and subsequent staining of gels with Coomassie brilliant blue R were performed by the procedure of Weber and Osborn (27). Cylindrical (5 × 110 mm) gels were 5% (w/v) acrylamide and 0.14% (w/v) *N,N'*-methylenebisacrylamide. Samples were prepared for electrophoresis by heating to 100°C in 10 mM Na-phosphate (pH 7.1–7.2), 0.79 M 2-mercaptoethanol, 2% (w/v) SDS, 32% (v/v) glycerol, and 0.0015% (w/v) bromphenol blue for 5 min.

Analytical isoelectric focusing was carried out in 4% (w/v) polyacrylamide gels as described by Righetti and Drysdale (24) and as performed previously (8). Gels were scanned without staining.

Amino Acid Analysis. Amino acid analyses were performed by the procedure of Moore and Stein (11). Determinations were made for three different HCOOH-eluted, immunopurified zucchini phytochrome samples with two different analyzers, each of which was independently calibrated. The two analyzers yielded data that agreed closely for all amino acids except proline. Combined data are presented. Methionine and ½-cystine were determined after performic acid oxidation (7). Tryptophan was determined spectrophotometrically (6).

Absorbance Spectra. Absorbance spectra were recorded with a Shimadzu MPS-50L spectrophotometer (Shimadzu Seisakusho Ltd., Kyoto, Japan) after saturating irradiation with red (Balzer B-40, 663-nm interference filter, Liechtenstein) or far-red (Plexiglas FRF-700 cut-off filter, Westlake Plastic Co., Lenni, PA) light. Wavelength calibration was checked with a didymium filter, which was scanned either immediately before or immediately after the phytochrome spectra were recorded.

Antizucchini-Phytochrome Serum. Approximately 1 unit of brushite-purified zucchini phytochrome was precipitated with 20 ml antiundegraded-oat-phytochrome serum (15 for antioat-phytochrome serum preparation). The immunoprecipitate, which was bright blue, was collected by centrifugation; washed by resuspension and recentrifugation, twice with 131 mM NaCl containing 0.1% Triton X-100 and 0.1% sodium desoxycholate and once with 145 mM NaCl; suspended in 1.0 ml 0.1 M Na-phosphate (pH 7.8); mixed with 1.0 ml complete Freund's adjuvant; and injected into a rabbit, one-half intramuscularly and one-half subcutaneously. A second identical washed immunoprecipitate was injected 1 month later. The rabbit was bled and the serum prepared as described elsewhere (15).

Antizucchini-Phytochrome Immunoglobulins. Approximately 4 units of conventionally purified pea phytochrome (SAR = 0.18) were covalently linked to 2.7 g CNBr-activated agarose (8). Antizucchini-phytochrome immunoglobulins were immunopurified from the crude serum by a procedure slightly modified from that described by Hunt and Pratt (8). The immobilized pea phytochrome, which was used for the immunopurification of antizucchini-phytochrome immunoglobulins, was handled throughout in a syringe that was used as a small chromatography column. Buffers were at pH 7.8 rather than 7.5. The crude serum was repeatedly adsorbed until no more antiphytochrome immunoglobulins could be detected in the HCOOH eluate. During HCOOH elution of the immunoglobulins, the eluate of the pea phytochrome column was passed immediately through a Sephadex G-25 column to separate the immunoglobulins from the HCOOH.

The antizucchini-phytochrome immunocolumn used for the purification of dicotyledonous phytochrome (see above) was prepared by covalently linking 14 mg of the purified immunoglobulins to 1.7 g of CNBr-activated agarose (8).

Immunochemistry. Ouchterlony double immunodiffusion and immunoelectrophoresis were performed as before (12, 15).

RESULTS

Antizucchini-Phytochrome Serum and Immunoglobulins. Antiserum against undegraded oat phytochrome exhibits a weak but distinct reaction against zucchini phytochrome as assayed by Ouchterlony double immunodiffusion (Fig. 1). This cross-reaction, even though weak, permitted the immunoprecipitation of zucchini phytochrome by the antioat-phytochrome serum. After this immunoprecipitation, the antioat-phytochrome serum no longer exhibited reactivity against zucchini phytochrome (note the absence of a precipitation line between wells S' and Z) even though it still precipitated oat phytochrome (Fig. 1).

The antizucchini-phytochrome serum, which was prepared by injection of immunoprecipitates, exhibits a strong, but possibly nonspecific, reaction against zucchini phytochrome as assayed by immunoelectrophoresis (Fig. 2a).

An SDS-polyacrylamide gel scan after electrophoresis of the pea phytochrome used to immunopurify antizucchini-phytochrome immunoglobulins (Fig. 3) indicates that the predominant polypeptide is at about 117,000 daltons, the mobility of undegraded pea phytochrome (8, 28). The most abundant minor contaminant is estimated to be at about 26,000 daltons, just before the bromphenol blue front. The antizucchini-phytochrome immunoglobulins immunopurified by this immobilized pea phyto-

[4] Abbreviations: SAR, specific absorbance ratio, A_{667}/A_{280} with phytochrome as Pr.

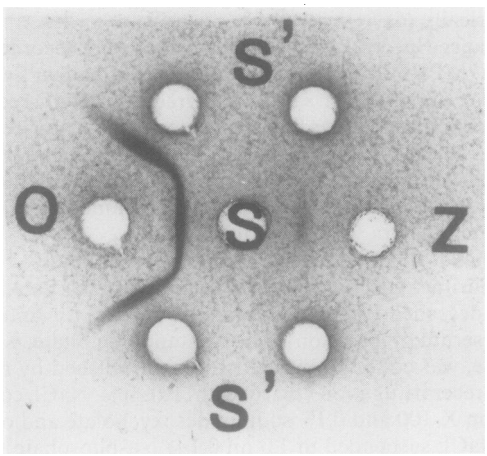

FIG. 1. Ouchterlony double immunodiffusion of crude antiundegraded-oat-phytochrome serum (S), or the same antiserum after immunoprecipitation of zucchini phytochrome (S'), against brushite-purified oat (O, SAR = 0.04) or brushite-purified zucchini (Z, SAR = 0.04) phytochrome. Antisera were used undiluted; phytochrome was used at a concentration of about 0.2 unit/ml. Precipitation lines were stained with Coomassie brilliant blue R.

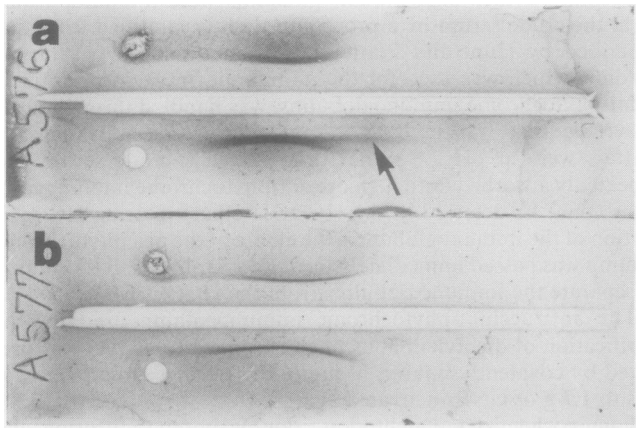

FIG. 2. Immunoelectrophoretic patterns obtained after electrophoresis of either immunopurified $MgCl_2$-eluted zucchini phytochrome (upper well in a and b, SAR not determined, about 1 unit/ml) or brushite-purified zucchini phytochrome (lower well in a and b, SAR = 0.04, 0.3 unit/ml) and subsequent diffusion against (a) undiluted crude antizucchini-phytochrome serum or (b) $MgCl_2$-eluted immunopurified antizucchini-phytochrome immunoglobulins (0.7 mg/ml). Precipitation lines were stained with Coomassie brilliant blue R.

chrome also exhibit a strong reaction against zucchini phytochrome as assayed by immunoelectrophoresis (Fig. 2b). The minor precipitation line (arrow in Fig. 2a) observed with the crude serum is, however, essentially absent when the trough is filled with the purified immunoglobulins.

Immunopurification of Dicotyledonous Phytochrome. Phytochrome was immunopurified by the antizucchini-phytochrome immunocolumn from brushite-purified pea and lettuce phytochrome preparations and from either crude or brushite-purified zucchini phytochrome preparations. $MgCl_2$-eluted zucchini phytochrome, which is both undenatured and undegraded, is quite pure as assayed by SDS-polyacrylamide gel electrophoresis and exhibits a monomer size of 121,000 daltons (Fig. 4b). HCOOH-eluted zucchini phytochrome is less homogeneous by the same criterion (Fig. 4a), presumably either because of the presence of contaminants or of proteolytic degradation products. Highly purified preparations of undegraded and undenatured pea phyto-

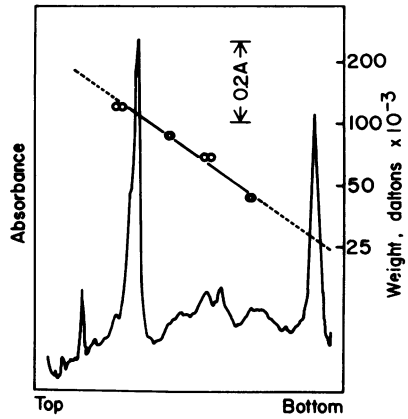

FIG. 3. Absorbance scan of an SDS-polyacrylamide gel after electrophoresis of about 10 μg protein from a partially purified pea phytochrome preparation (SAR = 0.18) and staining with Coomassie brilliant blue R. Size standards were BSA, monomer (66,000 daltons) and dimer; immunoglobulin G heavy chain (50,000 daltons), and ovalbumin (45,000 daltons).

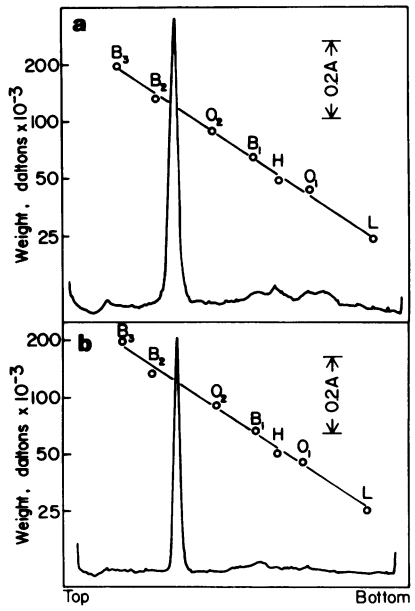

FIG. 4. Absorbance scans of Coomassie blue-stained, SDS-polyacrylamide gels after electrophoresis of (a) about 0.003 units of HCOOH-eluted immunopurified zucchini phytochrome (SAR = 0.56) or (b) about 0.003 units of $MgCl_2$-eluted immunopurified zucchini phytochrome. Size standards are the same as in Figure 3 except that BSA trimer, ovalbumin dimer, and immunoglobulin G light chain (25,000 daltons) could also be resolved.

chrome may also be obtained with this same immunocolumn (Fig. 5a).

Attempts to immunopurify lettuce phytochrome, however, led to severe difficulty, apparently with high endogenous protease activity. In the absence of protease inhibitors, we were unable to obtain lettuce phytochrome with an electrophoretic mobility near 120,000 daltons, even though immunoprecipitates prepared with the antizucchini-phytochrome serum were visibly blue. When protease inhibitors were used throughout the purification (see under "Materials and Methods") it was possible to obtain lettuce phytochrome samples in which the major polypeptide electrophoresed with a mobility equivalent to about 120,000 daltons (Fig. 5b). Even with the protease inhibitors smaller peptides were present in the immunopurified samples.

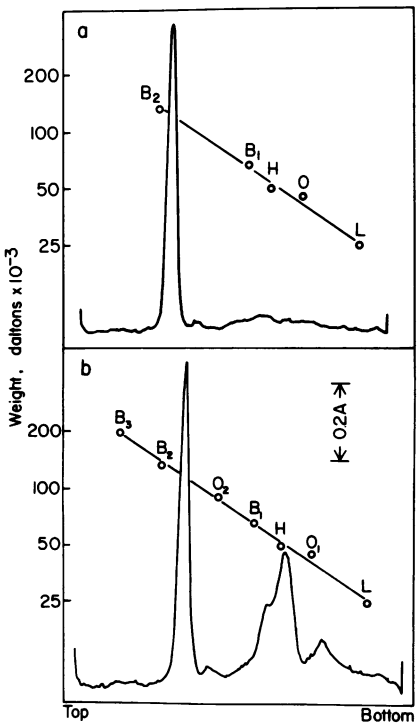

FIG. 5. Absorbance scans of Coomassie blue-stained, SDS-polyacrylamide gels after electrophoresis of (a) 0.006 units of $MgCl_2$-eluted immunopurified pea phytochrome (SAR = 0.54) or (b) 0.003 units of $MgCl_2$-eluted immunopurified lettuce phytochrome (SAR = 0.28). Size standards were the same as in Figure 4.

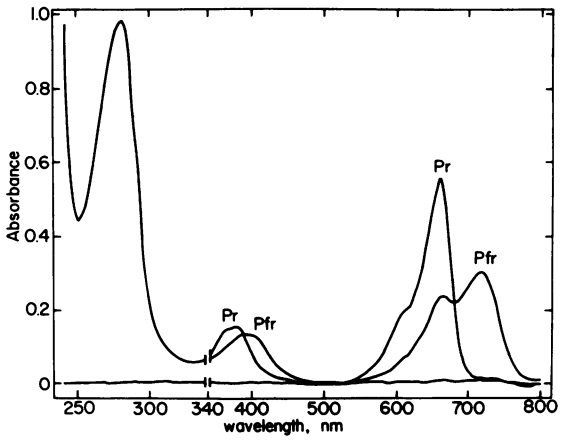

FIG. 6. Absorbance spectra of $MgCl_2$-eluted immunopurified zucchini phytochrome (SAR = 0.58) after saturating red (Pfr) or far-red (Pr) irradiation. Note that the wavelength expansions in the ultraviolet and visible regions differ.

Initial Characterization of Zucchini Phytochrome. Absorbance spectra of $MgCl_2$-eluted, immunopurified zucchini phytochrome (SAR = 0.58) exhibit maxima at 280, 382, and 668 nm for Pr, and at 280, ~395, 672, and 724 nm for Pfr (Fig. 6). The phytochrome was greater than 95% photoreversible but did tend to become turbid with time after conversion to Pfr. Absorbance spectra of comparably purified pea phytochrome were recorded only in the visible region (not shown) and were indistinguishable from those published earlier (see Fig. 2 in Pratt and Cundiff, 1975) for conventionally purified pea phytochrome.

Three phytochrome bands in the pH range 6.5 to 7.0 are observed after isoelectric focusing of $MgCl_2$-eluted, immunopurified zucchini phytochrome (Fig. 7). These bands are all assumed to be phytochrome because they absorb at 667 nm in the unstained gel and because they appear blue under white light. In addition, they are reversibly bleached by red (middle trace, Fig. 7) and far-red (upper trace, Fig. 7) irradiation, although one cannot be absolutely certain that all three bands undergo equivalent absorbance changes.

The electrophoretic mobility of undegraded zucchini phytochrome, under conditions that are nondenaturing and do not produce any molecular sieving, is similar to that of undegraded pea (Fig. 8) and undegraded oat (data not shown) phytochrome.

The amino acid content of undegraded HCOOH-eluted, immunopurified zucchini phytochrome is quite similar to that reported earlier for undegraded rye (22) and undegraded oat (9, 26) phytochrome (Table I). Because of the uniquely large difference in the value obtained for proline with the two amino acid analyzers that were used (95 with one analyzer, 41 with the other) no value is entered for this amino acid.

DISCUSSION

Antiserum and Immunoglobulins. The purification by conventional methods of a dicotyledonous phytochrome to a degree of homogeneity sufficient to produce a specific antiserum has proven exceptionally difficult (2; Pratt, unpublished observations). Similarly, immunopurification of a dicotyledonous phytochrome with an antioat-phytochrome immunocolumn (8) in quantity sufficient for antiserum production has also been impractical. We have thus

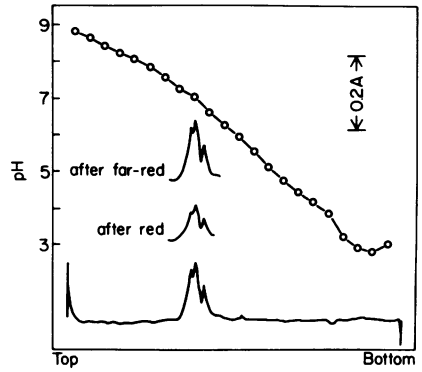

FIG. 7. Absorbance scans at 667 nm of an unstained polyacrylamide gel after isoelectric focusing of about 0.07 units of $MgCl_2$-eluted immunopurified zucchini phytochrome (SAR = 0.48). The first scan (complete, lower trace) was made prior to any actinic irradiation of the gel (focusing was performed under green light). The gel was then partially rescanned after red and then far-red irradiation. The pH profile of the same gel is shown.

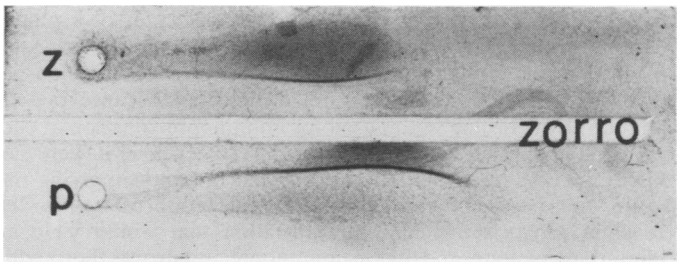

FIG. 8. Pattern obtained after electrophoresis of partially purified pea phytochrome (P) (SAR = 0.18; 0.59 unit/ml), and immunopurified 3 M $MgCl_2$-eluted zucchini phytochrome (Z) (SAR = not determined; about 1 unit/ml), and subsequent diffusion against immunopurified antizucchini-phytochrome immunoglobulins (Zorro, which was the name of the rabbit; 0.7 mg/ml). The plate was stained with Coomassie brilliant blue R.

Table I. *Amino Acid Analysis of HCOOH-Eluted Immunopurified Zucchini Phytochrome*

Data for zucchini phytochrome are compared to earlier analyses reported for undegraded oat (9) and undegraded rye (22) phytochrome. Entries are number of residues per approximately 120,000-dalton monomer.

Residue	Phytochrome		
	Zucchini	Oat	Rye
Lysine	64	64	58
Histidine	24	34	28
Arginine	48	51	47
Aspartic acid	110	118	104
Threonine	54[a]	38	46
Serine	77[a]	73	75
Glutamic acid	128	122	128
Proline	?[b]	45	88
Glycine	82	72	77
Alanine	79	93	110
½-Cystine	22[c]	27	26
Valine	72[d]	79	89
Methionine	28[e]	26	32
Isoleucine	59[d]	51	54
Leucine	120	119	111
Tyrosine	25	23	23
Phenylalanine	41	45	43
Tryptophan	8[f]	8	ND[g]

[a] Determined by extrapolation of 24-, 48-, and 72-h hydrolysates to 0 time.

[b] Not entered because of large variation between replicate measurements.

[c] From performic acid-oxidized sample only.

[d] From 72-h hydrolysates only.

[e] Average of both performic acid-oxidized sample and unmodified samples.

[f] Determined spectrophotometrically.

[g] Not determined.

depended upon the weak but adequate immunochemical cross-reaction between antioat-phytochrome serum and zucchini phytochrome (Fig. 1) to produce immunoprecipitates that could be used for antizucchini-phytochrome serum production. An optimal ratio of antioat-phytochrome serum to zucchini phytochrome was determined by a series of preliminary immunoprecipitations in which this ratio was varied. A rabbit would not be expected to produce antibodies against immunoglobulins obtained from another rabbit and even if it did these antibodies would be of little to no concern since it is safe to assume that plants do not contain any protein antigenically comparable to a rabbit immunoglobulin.

Immunoelectrophoretic analysis (Fig. 2a) of the crude serum indicates the existence of either a nonspecificity or of a phytochrome proteolytic degradation product (3, 5) in the brushite-purified zucchini phytochrome sample. The presence of a nonspecificity in the antiserum was not anticipated because (a) the antioat-phytochrome serum used for immunoprecipitation of zucchini phytochrome was highly specific, (b) even if it were not highly specific the probability is low that the antigen giving rise to this nonspecificity would also be found with brushite-purified zucchini phytochrome at a concentration that would yield an immunoprecipitate, and (c) the immunoprecipitate was thoroughly washed with detergents. Nevertheless, even if the antiserum is nonspecific, the immunoglobulins purified from it appear highly specific as indicated by the absence of additional precipitation lines after immunoelectrophoresis against brushite-purified zucchini phytochrome (Fig. 2b) and by the high degree of purity of phytochrome that can be immunopurified by these immunoglobulins (Figs. 4b and 5a).

Phytochrome Immunopurification. Because of the strong cross-reactions between different dicotyledonous phytochromes and antiserum against a single dicotyledonous phytochrome (Cordonnier and Pratt, in preparation), it is possible to immunopurify efficiently various dicotyledonous phytochromes (Figs. 4 and 5) with this single antizucchini-phytochrome immunocolumn. Conversely, it is also practical to utilize, as was done here, an immobilized phytochrome from one dicotyledonous plant to immunopurify immunoglobulins against phytochrome from another dicotyledonous plant.

Yields obtained here for the immunopurification of zucchini phytochrome, which were about 5 to 10% for the $MgCl_2$-eluted pool and about 30% additional in the HCOOH-eluted pool, were low compared to those obtained for oat phytochrome (8, 16). Attempts to improve upon these yields by changing elution conditions failed. Elution by saturated $MgCl_2$ led to the appearance of an additional peptide upon SDS-polyacrylamide gel electrophoresis with a size of about 200,000 daltons, which conceivably could have been a phytochrome dimer, without increasing the yield. Elution with thiocyanate or with $MgCl_2$ at lower pH did increase the yield but also resulted in spectral denaturation (1). By repetitive adsorption and 3 M $MgCl_2$ elution as described under "Materials and Methods," however, it was possible to increase the yield of $MgCl_2$-eluted phytochrome to 15% or more.

Initial Characterization of Zucchini Phytochrome. The 121,000-dalton monomer size of zucchini phytochrome (Fig. 4) is similar to that reported for rye (5), oat (2, 9, 13, 26), pea (8, 28) (Fig. 5a) and lettuce (Fig. 5b) phytochrome. It cannot be ascertained from the present data whether the smear of low molecular weight peptides evident in some preparations (*e.g.* Figs. 4a and 5b) represents contaminants or, since proteolysis of phytochrome is a common and serious problem in its purification (5), proteolytic degradation products.

Absorbance spectra of zucchini phytochrome are virtually identical to those reported for undegraded oat (8), rye (22), and pea (17, 28) phytochrome with two exceptions. First, the far-red absorbance maximum for Pfr is significantly greater than that observed for other undegraded phytochromes. While this observation might indicate that the proportion of phytochrome present as Pfr at photoequilibrium is higher for zucchini phytochrome, other explanations are also possible. Second, highly purified zucchini phytochrome has a SAR of about 0.58 (Fig. 4b), significantly lower than that observed for comparably purified oat (SAR = 0.87 [8]) and rye (SAR = 0.8 [23]) phytochrome. A lower SAR was also observed for highly purified pea phytochrome (SAR = 0.54, Fig. 5a). It seems that the SAR indicative of a completely pure phytochrome sample might vary from one plant species to the next, although it must still be emphasized that the SAR by itself is not a rigorous index of purity (13, 14).

Yamamoto *et al.* (29) reported a SAR for purified undegraded pea phytochrome of 0.88, considerably higher than the 0.54 observed here. It seems likely, based upon the gel scan presented here (Fig. 5a), that our pea phytochrome sample is more than about 60% pure. One possible explanation for this apparent discrepency is that immunopurified phytochrome might include phytochrome apoprotein that is free of chromophore.

The virtually identical tryptophan and tyrosine contents of zucchini and oat phytochrome (Table I) indicate that their ultraviolet extinction coefficients ought to be the same. The apparently reduced SAR for pure zucchini phytochrome presumably must result from either fewer chromophores, as alluded to above, or lower visible chromophore extinction.

The amino acid contents of zucchini, oat, and rye phytochromes are similar (Table I). The only differences that exceed both 20% and an absolute difference of 10 residues are those between oat and zucchini phytochromes with respect to threonine and between

rye and zucchini phytochromes with respect to alanine. The similarity in amino acid compositions is reflected in the similar net electric charges exhibited by immunoelectrophoresis (Fig. 8 [12]).

Zucchini phytochrome exhibits heterogeneity by isoelectric focusing (Fig. 7) as described previously for oat phytochrome (8). As for oat phytochrome, the cause of this heterogeneity, or even whether or not it might be artifactual, is not known. The pI of zucchini phytochrome of 6.5 to 7.0 is significantly higher than the 5.8 to 6.4 observed for oat phytochrome. This difference was confirmed by focusing immunopurified undegraded oat phytochrome at the same time as the zucchini phytochrome that yielded the results shown here. Although their similar amino acid compositions (Table I) might indicate that oat and zucchini phytochrome should have a similar pI range, the observed difference is not surprising for at least two reasons. First, even though the number of glutamic and aspartic acids are virtually the same (238 *vs.* 240), the proportions that are present as glutamine and asparagine prior to hydrolysis may well be considerably different. Second, the extent of posttranslational modification of the two phytochromes (*e.g.* via phosphorylation [9]) might well differ. This higher pI for zucchini phytochrome must be taken into account when working with the molecule *in vitro*. For example, many proteins become less soluble near their pI. Consequently, observations concerning light-induced pelletability of zucchini phytochrome, which are typically made at or near a pH of 7, must be interpreted with appropriate caution. In addition, this obvious difference between oat and zucchini phytochrome reinforces the need to be cautious in general when extrapolating data obtained by study of one phytochrome species to phytochrome from another.

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