Immunochemical detection with rabbit polyclonal and mouse monoclonal antibodies of different pools of phytochrome from etiolated and green Avena shoots

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Abstract. While two monoclonal antibodies directed to phytochrome from etiolated oat (Avena sativa L.) shoots can precipitate up to about 30% of the photoreversible phytochrome isolated from green oat shoots, most precipitate little or none at all. These results are consistent with a report by J.G. Tokuhisa and P.H. Quail (1983, Plant Physiol. 72, Suppl., 85), according to which polyclonal rabbit antibodies directed to phytochrome from etiolated oat shoots bind only a small fraction of the phytochrome obtained from green oat shoots. The immunoprecipitation data reported here indicate that essentially all phytochrome isolated from green oat shoots is distinct from that obtained from etiolated oat shoots. The data indicate further that phytochrome from green oat shoots might itself be composed of two or more immunologically distinct populations, each of which is distinct from phytochrome from etiolated shoots. Phytochrome isolated from light-grown, but norflurazon-bleached oat shoots is like that isolated from green oat shoots. When light-grown, green oat seedlings are kept in darkness for 48 h, however, much, if not all, of the phytochrome that reaccumulates is like that from etiolated oat shoots. Neither modification during purification from green oat shoots of phytochrome like that from etiolated oat shoots, nor non-specific interference by substances in extracts of green oat shoots, can explain the inability of antibodies to recognize phytochrome isolated from green oat shoots. Immunopurified polyclonal rabbit antibodies to phytochrome from etiolated pea (Pisum sativum L.) shoots precipitate more than 95% of the photoreversible phytochrome obtained from etiolated pea shoots, while no more than 75% of the pigment is precipitated when phytochrome is isolated from green pea shoots. These data indicate in preliminary fashion that an immunochemically unique pool of phytochrome might also be present in extracts of green pea shoots.

Key words: Avena (phytochrome) – Enzyme-linked immunosorbent assay (ELISA) – Immunoprecipitation – Monoclonal antibody – Phytochrome from green and etiolated tissue – Pisum (phytochrome).

Introduction

A red/far-red reversible reaction mediated by phytochrome in light-grown, green plants was first reported by Borthwick et al. (1952a), soon after the original discovery of a physiological response mediated by the same pigment (Borthwick et al. 1952b). Nevertheless, little work has been done with phytochrome in green plants. This situation arose primarily for two reasons. Firstly, green plants have relatively little phytochrome (Lane et al. 1963; Taylor and Bonner 1967; Hunt and Pratt 1979b; Shimazaki et al. 1981). Secondly, chlorophyll interferes with all of the widely used spectral assays for phytochrome (see Pratt 1983 for review).

Lane et al. (1963) first reported that a difference spectrum between the red- and far-red-absorbing forms of phytochrome (Pr and Pfr, respectively) in extracts of a green plant (spinach) was not significantly different from that reported previously for phytochrome in etiolated plants. A similar observation has more recently been made for

Abbreviations: ELISA = enzyme-linked immunosorbent assay; mU = milliunit; Pfr = far-red-absorbing form of phytochrome; Pr = red-absorbing form of phytochrome
phytochrome from light-grown, green pea shoots as well (Shimazaki et al. 1981). Phytochrome preparations from green and etiolated pea tissues also exhibited identical immunchemical characteristics as assayed by Ouchterlony double diffusion and immunoelectrophoresis (Shimazaki et al. 1981).

In contrast to these results obtained with phytochrome from green spinach and green peas, Tokuhisa and Quail (1983) observed that a difference spectrum for phytochrome isolated from green oat shoots was different from a comparable spectrum for phytochrome from etiolated oat shoots. Furthermore, they reported that polyclonal rabbit antibodies directed to phytochrome from etiolated oat shoots bind at most only 30% of the phytochrome obtained from green oat shoots. Consistent with these observations, Shimazaki et al. (1983) found that an enzyme-linked immunosorbent assay (ELISA), which uses polyclonal rabbit and monoclonal mouse antibodies directed to phytochrome from etiolated oat shoots, detected a decreasing proportion of spectrophotometrically assayable phytochrome as etiolated seedlings were kept for increasing time in white light. These data also indicated that green oat shoots might contain a phytochrome pool that was antigenically distinct from that present in etiolated oat shoots.

We describe here a partial purification of phytochrome from light-grown, green oat shoots. Phytochrome preparations from both green and etiolated oat shoots are compared in terms of immunchemical reactivity with polyclonal rabbit and monoclonal mouse antibodies, all of which were directed to phytochrome from etiolated oat shoots. Emphasis is given to experiments designed to determine whether the observed immunchemical differences might be artifactual. Finally, an initial experiment that is intended to determine whether a comparable difference might exist for phytochrome from a dicotyledonous plant (pea) is also reported.

Materials and methods

Plants. Etiolated oats (Arena sativa L., cv. Garry; seeds from Whitney-Dickinson Seeds, Buffalo, N.Y., USA) and etiolated peas (Pisum sativum L., cv. Alaska; Leatherman's Seed Co., Canton, O., USA) were grown in total darkness at 25 °C and harvested as described in Pratt (1973). Green oats and green peas were grown in a greenhouse under an approx. 12:12-h dark-light cycle. Green shoots were harvested in daylight at the end of the light period from 11-d-old oat and 9-d-old pea seedlings. Some 11-d-old green oat seedlings were transferred to darkness and their shoots were harvested after 48 h of dark incubation at 25°C. To obtain light-grown, achlorophyllous oat shoots, seeds before planting were imbibed for 1 h in 0.2 mM norflurazon (Sandoz 9789, 4-chloro-5-(methylamino)-2-(α-α-α-trifluoro-m-tolyl)-3(2H)-pyridazinone), which was provided by Dr. Gerald Deitzer, Smithsonian Radiation Biology Laboratory, Rockville, Md., USA (see Jabben and Deitzer 1978a). Otherwise, these herbicide-treated oats were handled as the green oats. Harvested tissue was stored in darkness at −20°C prior to extraction.

Isolation of phytochrome. Oat phytochrome was partially purified by poly(ethyleneimine) and ammonium-sulfate fractionation and by hydroxyapatite chromatography according to the methods of Vierstra and Quail (1983). Modifications to their procedures were as follows: (i) frozen tissue was used for extraction; (ii) extraction buffer, which contained 50% ethylene glycol, was chilled to −20°C before use; (iii) in addition to 4 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine and 10 mM ε-aminocaproic acid, which also inhibit phytochrome proteolysis in crude extracts (Cordonnier and Pratt 1983), were present in the extraction buffer; (iv) crude homogenates of etiolated oats, or supernatants obtained after clarification of light-grown oat extracts in the presence of poly(ethyleneimine) (Vierstra and Quail 1983), were irradiated with Gro-lux lamps (Sylvania, Fall River, Mass., USA), which produce essentially the same photostationary state as monochromatic red light; (v) a 2.4-cm diameter, 10-cm (for extracts of etiolated shoots) or 20-cm (for extracts of light-grown shoots) long column of hydroxyapatite (prepared according to Siegelman et al. 1965) was used for purification of phytochrome from 1 kg tissue; and (vi) phytochrome was eluted from the hydroxyapatite column with a linear gradient formed from 150 ml each of 5 and 200 mM potassium phosphate, 5 mM ethylenediaminetetraacetic acid (EDTA), 14 mM 2-mercaptoethanol, pH 7.8. Phytochrome isolated from etiolated oat shoots was predominantly 124000 dalton in size, based upon the following criteria (Litts et al. 1983; Vierstra and Quail 1982, 1983): (i) the absence of appreciable reversion even in the presence of 5 mM dithionite; (ii) a longer wavelength maximum for Pfr absorbance; and (iii) mobility during sodium dodecyl sulfate, polyacrylamide gel electrophoresis in 5% gels. For this last criterion, partially purified phytochrome was compared by immunoblotting to phytochrome extracted from lyophilized, etiolated oat shoots directly into boiling sodium dodecyl sulfate sample buffer (Cordonnier et al. 1985). Unless specifically noted otherwise, all oat phytochrome was purified through the hydroxyapatite step. Table 1 provides a summary of the different kinds of oat phytochrome preparations that were used.

Pea phytochrome was purified from etiolated and green shoots by brushite, diethylaminoethyl-agarose (DEAE-Bio-Gel A; Bio-Rad, Richmond, Cal., USA) and Sephacryl S-300 (Pharmacia, Uppsala, Sweden) chromatography as described in Hunt and Pratt (1979a) and Pratt (1984).

Phytochrome was kept at 0−4°C during isolation and purification and handled under green light (Pratt 1984), unless otherwise noted. Phytochrome preparations were stored at −80°C in 0.1 M sodium phosphate, 1 mM EDTA, pH 7.8.

Spectrophotometric measurement of phytochrome. Absorbance and phototransformation difference spectra of phytochrome were measured with clarified solutions using a split-beam spectrophotometer (Hitachi model 320, obtained from Perkin Elmer, Norwalk, Conn., USA). Samples were maintained at 2−4°C by circulating ice-cold water through the cuvette holder.Dry air was used to avoid accumulation of moisture on the cuvette surfaces.

Phytochrome photoreversibility (Butler and Lane 1965) was measured with a custom-built dual-wavelength spectrophotometer (Pratt et al. 1985). Measuring wavelengths were chosen according to peak wavelengths in corresponding phototransform-