Phytochrome in Cultured Wild Carrot Tissue. I. Synthesis

Donald F. Wetherell

Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268

Received July 15, 1969.

Abstract. Stable levels of P_{PR} occur in light-grown suspension cultures of wild carrot. Upon darkening such tissue, P_2 accumulates until the high P_2 level characteristics of dark-grown tissue is reached. Data are compatible with the idea of an equilibrium between processes of synthesis and destruction in tissue grown in the light. Reappearance of phytochrome also follows light-induced pigment destruction in dark-grown tissue. The rate of P_2 appearance is similar in both cases. The rate of disappearance of P_{PR}, however, is slower in light-grown tissue than in dark-grown tissue.

An earlier communication (7) called attention to the potential value of cultured tissues for the study of phytochrome physiology. The advantages of wild carrot tissue grown in suspension culture reside in its highly meristematic nature, high phytochrome levels, homogeneity, lack of chlorophyll, and capacity for adventive embryogenesis.

In this report the phytochrome levels of light-grown tissue are measured and the synthesis of new phytochrome is partially characterized. The term "synthesis" is used here to denote an increase in photoreversible phytochrome. The extent to which this is due to true de novo synthesis is not known.

Materials and Methods

The tissue used in these experiments was obtained originally from petiole explants of the wild carrot Daucus carota as described by Halperin and Wetherell (3). It has been subcultured for 5 years as a suspension culture in the auxin-containing medium described below.

The standard culture medium contains the inorganic salts according to Lin and Stauba (5) except that KNO_3 is increased to 4.0 g/l and NH_4Cl is substituted for NH_4NO_3 and supplied at 0.54 g/l. Iron is supplied as the ferrous EDTA chelate at a concentration of 5 \times 10^{-6} M. Thiamine at 3 mg/l and sucrose at 20 mg/l complete the medium which is finally adjusted to pH 5.6 and sterilized by autoclaving. When present, the auxin 2,4-D is added at a concentration of 2.3 \times 10^{-6} M.

Screw-topped test tubes and bottles of various sizes filled to 20% of their volume with media, are rotated horizontally at 10 rpm, 25°C, and in a cool white fluorescent illuminance of approximately 60 ft-c unless otherwise noted.

Red irradiation was obtained from cool white fluorescent lamps filtered through red plexiglass. Phytochrome measurements were made with a Ratio-spect\textsuperscript{a} dual wavelength spectrophotometer equipped with interference and supplementary filters giving peak transmission at approximately 665 and 725 nm. Tissue was pelleted and transferred to pre-chilled 6 mm ID cylindrical metal cuvettes (2) working under a green safelight (8). Sample size was routinely 0.2 cc of centrifuge-packed tissue (150 x g). Packed tissue (1 ml) has a fresh weight of 550 mg and a dry weight of approximately 8% of this value. Filled cuvettes were kept in crushed ice until and during measurements. Many of the measurements included in this work are made near the limit of resolution of the equipment. For this reason, triplicate and frequently quadruplicate samples were taken and at least 2 measurements made on each sample. The use of a Sorensen Model 1500 voltage regulator added to the stability and reproducibility of the measurements. In this way it was possible to hold the standard error to 35% or less for \Delta(\Delta OD) values as low as 0.004. Reliability of data was also increased by replication of experiments.

Results

Fig. 1 shows that apparent phytochrome synthesis in darkened light-grown tissue proceeds at an approximately linear rate until a pigment level characteristic of dark-grown tissue is reached. Because of the age and high tissue density of these cultures, growth is slow. Volume of packed tissue per volume of culture increased by only 40% during the 75 hr period plotted in Fig. 1. In 5 similar experiments the rate of synthesis varied from 9 \times 10^{-4} to 18 \times 10^{-4} \Delta(\Delta OD) per 0.2 cc of packed tissue per 24 hr, but the graph was always linear and there was no indication of a lag in the beginning of

---

1 Work supported in part by Grant GB-5624 from the National Science Foundation and the University of Connecticut Research Foundation.

2 Model R-2 Agricultural Specialties Company, Hyattsville, Maryland, U.S.A.
synthesis. Fig. 2 presents similar data taken over a shorter time course and includes P_{FR} disappearance. A lag of 3 to 4 hr was observed in 3 experiments where this latter parameter was measured.

The wide range of rates of apparent phytochrome synthesis observed, suggested that culture age might be an important factor. Table I presents data confirming this idea. In additional similar experiments rates as high as $18 \times 10^{-8} \Delta(\Delta OD)$ per 0.2 cc per 24 hr were recorded, however, these periods of rapid synthesis were short-lived. Table II represents an attempt to find ways of enhancing or inhibiting apparent synthesis using ideas adopted from the literature. Apart from the clear restriction of synthesis in the absence of sucrose, no significant changes were observed.

Table I. Culture Age, Growth, and Rate of Apparent Phytochrome Synthesis in Darkened Light-grown Wild Carrot Tissue

<table>
<thead>
<tr>
<th>Age in days</th>
<th>Growth (mm$^3$/ml)</th>
<th>Apparent synthesis $\Delta(\Delta OD) \times 1000$ per 0.2 cc per 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13</td>
<td>3.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>6.5</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>14.0</td>
</tr>
<tr>
<td>10</td>
<td>65</td>
<td>17</td>
</tr>
<tr>
<td>14</td>
<td>80</td>
<td>2.0</td>
</tr>
<tr>
<td>16</td>
<td>135</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Fig. 1. Apparent synthesis of phytochrome in a light-grown suspension culture of wild carrot tissue during 75 hr of darkness. Tissue volume has increased by 40% during this period.

Table II. Apparent Phytochrome Synthesis in Suspension Cultures of Wild Carrot Tissue

Tissue was incubated with (MS) and without (M) sucrose and in the presence of 2,4-D at $2 \times 10^{-8}$ M (MSD) or naphthaleneacetic acid at $2 \times 10^{-8}$ M (MSN) or $2 \times 10^{-6}$ M 2,4-D and $1 \times 10^{-2}$ M glycine and succinic acid (MSDG) for the period of time indicated. Sample size is 0.2 cc of packed tissue. Initial $\Delta(\Delta OD)$ is $2.5 \times 10^3$.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>$\Delta(\Delta OD) \times 1000$ at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 hr</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
</tr>
<tr>
<td>MS</td>
<td>7</td>
</tr>
<tr>
<td>MSD</td>
<td>8</td>
</tr>
<tr>
<td>MSN</td>
<td>7</td>
</tr>
<tr>
<td>MSDG</td>
<td>8</td>
</tr>
</tbody>
</table>

Table III presents 1 of 3 experiments conducted to measure apparent synthesis in dark-grown tissue in which the normally high phytochrome levels have been reduced by red light irradiations. Little or no new synthesis occurred when pigment level was reduced to roughly 50% of initial levels, however a period of strong synthesis followed reductions to approximately 15%.

Discussion

Light-grown wild carrot tissue contains phytochrome in the P_{FR} state at levels ranging from $1 \times 10^{-8}$ to $5 \times 10^{-3}$ $\Delta(\Delta OD)$ per 0.2 cc of packed tissue. Accurate measurements of relatively low pigment concentrations are possible in this undifferentiated auxin-grown tissue because it lacks pigments which interfere with differential optical density.

Fig. 2. Apparent synthesis of phytochrome and P_{FR} disappearance during a 23 hr dark treatment of light-grown tissue of wild carrot.
measurements at the absorption maxima of phytochrome. Darkening of light-grown tissue leads to the appearance and accumulation of photoreversible phytochrome in the \( P_R \) form. Accumulation continues at a linear rate until the level characteristic of the dark-grown material is reached. Maximum levels for dark-grown tissue may reach 10 times the maximum levels of light-grown tissue.

Rates of synthesis vary greatly with the physiological age of the culture. Table I shows a brief sharp rise in the rate of synthesis 3 days after transfer to fresh media. This rise correlates with the beginning of the period of rapid increase in cell mass. Torrey et al. (6) and others have shown this to be also a period of high rates of cell division in contrast to the low rates which occur during the major part of the period of growth. Throughout subsequent mainly expansive growth, rates of synthesis are low and fall to unmeasurable levels in dense older cultures. These data suggest a relationship between cell division and the appearance of photoreversible phytochrome.

Clarkson and Hillman reported a strong inhibition of apparent phytochrome synthesis upon the addition of IAA, NAA, or 2,4-D auxins to pea stem segments (1). These auxins were applied at a concentration \((1 \times 10^{-4} \text{ M})\) which did not inhibit growth. Choosing concentrations of NAA and 2,4-D which were threshold with respect to growth inhibition, we were unable to confirm this effect in our material (See Table II). The rapidly expanding cells of the apical hook region used in their experiments and the expanding cells of our cultured tissue should be comparable. It, therefore, seems unlikely that a consistent relationship between auxin levels and phytochrome synthesis exists.

Several experiments were run to compare the reappearance of phytochrome following red light-induced destruction in dark-grown tissue with that in darkened light-grown tissue. Phytochrome levels are lowered nearly 50% by a single saturating irradiation with red light. They can be further lowered to the level characteristic of light-grown tissue by several such irradiations spaced up to several hr apart. Table III shows that the rate and amount of resynthesis appears related to the extent of phytochrome depletion. Clarkson and Hillman have observed such a relationship in pea stem segments and have suggested that synthesis may be linked to phytochrome level by a feedback regulatory system (4). The maximum rate of resynthesis in dark-grown material is comparable to that in darkened light-grown material. In these experiments re-synthesis did not attain the levels of phytochrome of the initial dark-grown tissue. However, this was due primarily to the fact that the cultures had become dense and slow growing by this time.

In spite of large starch reserves, phytochrome synthesis is dependent upon exogenous sucrose supplies. Table II and similar experiments show that little or no synthesis occurs in sucrose-free media. Growth also stops soon after the removal of sucrose, however, the tissue remains viable and starch reserves disappear.

Phytochrome accumulation occurs in the \( P_R \) form. There appears to be no lag in the early period of accumulation. \( P_{PR} \) disappearance on the other hand, appears to exhibit a lag (Fig. 2) and then proceeds at a rate considerably slower than the rate of disappearance from dark-grown tissue (7). These data support the idea advanced by Clarkson and Hillman that the relatively constant level of phytochrome found in light-grown tissue may be due to an equilibrium between processes of destruction and synthesis (1). One would expect a measurable lag at the beginning of the synthesis period if this process was not already operating. Conversely, the apparent lag in \( P_{PR} \) disappearance might be interpreted to mean that the pigment is light stable. However, when tissue was incubated in sucrose-free media in the light, the phytochrome level dropped from a \( \Delta(\Delta \text{OD}) \times 10^8 \) of 4 to 0 in 14 hr. This would be expected if \( P_{PR} \) synthesis was reduced by a shortage of sucrose (table II, treatment M) and if \( P_{PR} \) destruction continued at an unrestricted rate. It seems more likely that the lag represents a period of readjustment of \( P_{PR} \) destruction rate related to the light-dark transition.

In summary, measurable levels of phytochrome in the \( P_{PR} \) state persist in light-grown tissue. Data indicate that these levels are maintained by balanced new synthesis and destruction. Accumulation of \( P_R \) phytochrome proceeds in darkness if substrate levels are adequate. Accumulation rate varies with the physiological age of the tissue and pigment levels characteristic of dark-grown tissue are eventually reached. \( P_R \) accumulation appears similar in both light-grown and dark-grown tissue, however, \( P_{PR} \) destruction is much slower in the former material.
Literature Cited


