Light-dependent cytochrome *P*-450 changes in mung beans (*Phaseolus aureus*)

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Maximum concentrations of microsomal cytochrome *P*-450 are present in 3–4-day-old mung beans (*Phaseolus aureus*). On illumination of dark-grown seedlings, cytochrome *P*-450 and later cytochrome *P*-450 undergo a rapid decrease in concentration *in vivo*, with an apparent half-time of about 6 h. Conversely light-grown seedlings, transferred to darkness, show a slow accumulation of cytochrome *P*-450, doubling time of about 30 h, with a later accumulation of cytochrome *P*-420. Microsomal cytochromes b_{559} , $b_{560.5}$ and $b_{562.5}$ do not significantly alter on light–dark transitions. Possible functions for dark-induced cytochrome *P*-450 are discussed.

The functions of plant microsomal cytochrome *P*-450 include mono-oxygenase hydroxylations of a number of relatively water-insoluble products of terpene and cinnamate (flavonoid) metabolism (see Reichhart *et al.*, 1980a) and the desaturation of fatty acids (Soliday & Kolattukudy, 1977). Such functions are analogous to certain of those occurring in mammalian cytochrome *P*-450-mediated reactions (De Matteis, 1980). As with animal systems, induction of cytochrome *P*-450 by xenobiotics has been reported in higher plants (Reichhart *et al.*, 1980a,b). Of perhaps more physiological relevance are the reports of cytochrome *P*-450 induction in plants on wounding (Rich & Lamb, 1977; Reichhart *et al.*, 1980a), though functions for such induced forms of the cytochrome have not yet been fully resolved.

The activity of one type of cytochrome *P*-450 mono-oxygenase reaction, *trans*-cinnamate hydroxylation, is increased as a phytochrome-mediated response to far-red light in pea seedlings (Benveniste *et al.*, 1978), although no significant increase in the concentration of cytochrome *P*-450, nor microsomal cytochrome b_{559} (on a per gram fresh weight basis) accompanied the increased hydroxylation activity. Apart from pea seedlings, there is no evidence available to show if light has any effect on induction of plant cytochrome *P*-450. We have previously shown that mung-bean (*Phaseolus aureus*) seedlings grown in darkness for up to 7 days contain some two to three times higher concentrations of cytochrome *P*-450 than do seedlings grown under continuous illumination (Hendry *et al.*, 1981). Changes in cytochrome *P*-450 concentration

were related to age in both light- and dark-grown tissue. The evidence suggested that, in mung beans, there is a short-term requirement for cytochrome *P*-450 that may become largely redundant as the seedling achieves photosynthetic competence. What triggers this redundancy is not clear. Certainly there is no evidence from extensive mammalian studies that light has any immediate direct effect on cytochrome *P*-450 metabolism.

We have therefore investigated the effect of exposure to light, or darkness, on the concentration of plant microsomal cytochrome *P*-450, its probable degradation product cytochrome *P*-420, and on microsomal *b*-cytochromes. To avoid the considerable variation that occurs in endogenous microsomal cytochromes with age, we have restricted our studies to 72-h-old seedlings.

Materials and methods

Materials

Mung-bean seeds were purchased locally. Analytical-grade chemicals were supplied by BDH Chemicals, Poole, Dorset, U.K. Beef liver catalase and horseradish peroxidase were purchased from Boehringer, Mannheim, Germany.

Isolation of microsomal fraction and cytochrome assays

Mung beans were imbibed in water for 18 h, sown on dampened paper towels and grown in darkness or light (from 60 W tungsten bulbs, 0.5 m above seedling height) at 20–23°C. During the 72 h growth

period (from time of sowing), batches of seedlings were transferred to light or darkness for various periods. Extraction and isolation of microsomal fraction ('microsomes') from 120g fresh wt. of seedlings was by previously published methods (Hendry *et al.*, 1981) as were the assays for microsomal cytochromes *b*, *P*-450 and *P*-420. We define *P*-420 as the pigment that in the ferric state binds CO with an absorbance maximum at 422 nm. This is generally considered to be a degradation product of cytochrome *P*-450 (De Matteis, 1980), although other haem pigments may form a CO complex at this wavelength. Contamination by organelle cytochrome oxidase, cytochromes *c* and *f* was monitored in all preparations and in total found not to exceed 2–3% of the sum of microsomal cytochromes. Peroxidase and catalase contamination of microsomes was assayed by measuring their CO-binding β -band difference spectra (λ_{max} , 535 nm) compared with cytochrome *P*-450/*P*-420 CO-binding β -band difference spectra (λ_{max} , 542 nm), such contamination being barely detectable.

Microsomes were resuspended to a protein concentration of about 12–15 mg/ml. Proteins were determined by the method of Bramhall *et al.* (1969). Chlorophylls were extracted and determined by the method of Arnon (1949).

Results

Light-mediated cytochrome *P*-450 changes

Fig. 1 shows the effect on cytochromes *P*-450 and *P*-420 on illuminating dark-grown seedlings for various times of the 72 h growth period. Within 3 h of exposure, cytochrome *P*-450 concentrations diminish. The cytochrome *P*-450 content declines in a biphasic pattern. A rapid disappearance of cytochrome *P*-450 occurs in the first 9 h (53% loss), followed by a slower decline over the succeeding 60 h (further 60% loss). The rapid phase gives an apparent half-time for cytochrome *P*-450 of about 6 h. The probable product of degradation, cytochrome *P*-420, accumulates briefly (0–3 h), then also undergoes a light-induced decrease with rapid depletion occurring between 3 and 12 h (Fig. 1). Detectable chlorophyll synthesis occurs within 3 h of illumination (inset, Fig. 1).

Microsomal protein concentrations in seedlings (0.8–1.0 mg/g fresh wt.) showed small changes on illumination of dark-grown tissue ($\pm 10\%$). However, an alternative indicator of microsomal membrane concentration is cytochrome *b*₅₅₉. Total *b* cytochromes do not significantly alter in amount with light or dark treatment. Illumination caused relatively small decreases in total microsomal *b* cytochromes from 0.33 nmol/mg of protein (0 h of light) to 0.28 nmol/mg of protein (6 h) to 0.31 nmol/mg of protein (24 h). We have previously shown that

Fig. 1. Effect of light on cytochrome *P*-450 (●) and cytochrome *P*-420 (○) concentrations in microsomes from dark-grown mung beans

Germinating mung beans were grown for 72 h in darkness or in light before isolation of microsomes. Cytochrome *P*-450 and cytochrome *P*-420 concentrations were determined by their dithionite-reduced-plus-CO-minus-dithionite-reduced difference spectra. S.E.M. values were calculated for $n = 16$ –18 (0 and 72 h) and $n = 4$ –5 (3–36 h). The inset shows the rate of chlorophyll formation in intact seedlings.

about 80% of the total microsomal *b* cytochromes is composed of cytochrome *b*₅₅₉ [$E_{m7.0} - 45$ mV ($E_{m7.0}$ is the oxidation-reduction mid-point potential determined at pH 7.0)], the remainder consisting of small amounts of cytochrome *b*_{560.5} ($E_{m7.0} + 49$ mV) and cytochrome *b*_{562.5} ($E_{m7.0} + 105$ mV) (Hendry *et al.*, 1981). The concentrations of these last two cytochromes were also unaffected by illumination. Fig. 2 shows the effect of illumination on the ratio of cytochromes *P*-450 and *P*-420 to microsomal cytochrome *b*. The light-induced decrease in both *P*-cytochrome concentrations is largely confined to the first 24 h of illumination, with an apparent half-time of about 8 h.

Dark-mediated cytochrome *P*-450 accumulation

Light-grown seedlings maintain relatively low concentrations of cytochrome *P*-450 even after 48 h of growth (Hendry *et al.*, 1981). Such seedlings, transferred to the dark, show a slow recovery with cytochrome *P*-450 net synthesis. Fig. 3 shows the

Fig. 2. Ratio of cytochrome *P*-450 (●), cytochrome *P*-420 (○) and total *P*-cytochrome (▲) concentrations to cytochrome *b* concentration in microsomes from 72 h-old mung beans exposed to light

Germinating mung beans were grown for 72 h in darkness or in light before isolation of microsomal cytochromes. Determinations of cytochrome *P*-450 and cytochrome *P*-420 concentrations were as in Fig. 1. Total *b* cytochromes from the same samples were measured by their oxidized-minus-reduced difference spectra. Ratios were calculated from the means of the values recorded in Fig. 1

Fig. 3. Effect of darkness on cytochrome *P*-450 (●), cytochrome *P*-420 (○) and total *P* cytochrome (▲) concentrations in microsomes from light-grown mung beans

Germinating mung beans were grown for 72 h in light or in darkness before isolation of microsomes. Cytochrome *P*-450 and cytochrome *P*-420 concentrations were determined as in Fig. 1. S.E.M. values were calculated for $n = 18-21$ (0 and 72 h) and $n = 4-7$ (12-48 h).

effect of transferring 72 h-old light-grown seedlings to darkness for various periods before microsomal isolation. Cytochrome *P*-450 concentrations (per mg of microsomal protein) increase, relatively slowly, over 48 h. The overall effect of darkness was to bring about net synthesis of cytochrome *P*-450, the concentration of which increased by 320% (0.010 to 0.032 nmol) with a doubling time of net synthesis of about 30 h. Cytochrome *P*-420 concentrations, however, did not increase until the tissue had been in darkness for 24 h, further increases continuing with sustained darkness.

Dark treatment produced no significant change in total microsomal cytochrome *b* content, the small variation from control (0 h of darkness, 0.46 nmol/mg of protein) was 0.53 nmol/mg of protein (24 h), 0.47 nmol/mg of protein (36 h) and 0.49 nmol/mg of protein (48 h). When expressed as cytochrome *P*-450 or *P*-420/cytochrome *b* ratios, the results of dark treatment (Fig. 4) emphasize the slow accumulation of cytochrome *P*-450 with a doubling time of about 40 h.

Attempts were made to show light- or dark-mediated changes in microsomal cytochrome *P*-450 using younger or older seedlings. Tissue 48 h or 90 h old showed similar, but less pronounced, effects to those presented above. However, 5-7-day-old seedlings showed little or no dark-mediated resynthesis and a slightly faster rate of light-mediated degradation. The endogenous cytochrome *P*-450 concentrations (Fig. 5) rapidly diminish in both light- and dark-grown seedlings after day 4. This decline appears to be irreversible, at least over 36 h.

Conclusions

When dark-grown seedlings are exposed to light during the 3 days of growth there is significant decrease in cytochrome *P*-450 concentration. The biphasic net decrease shows a period of rapid breakdown with a half-time of about 6 h (calculated per mg of microsomal protein) or 8 h (calculated per unit of microsomal cytochrome *b*). The probable

Fig. 4. Ratio of cytochrome P-450 (●), cytochrome P-420 (○) and total P cytochrome (▲) to cytochrome b in microsomes from 72h-old mung beans exposed to darkness

Germinating mung beans were grown for 72h in light or in darkness before isolation of the microsomal cytochromes. Determination of cytochromes P-450, P-420 and b were as in Figs. 1 and 2. Ratios were calculated from the means of the values in Fig. 3.

Fig. 5. Changes in the concentrations of cytochrome P-450 with age in microsomes from dark-grown (●) and light-grown (○) mung beans

Determination of cytochrome P-450 was from the dithionite-reduced-plus-CO-minus-dithionite-reduced difference spectra for 24 samples.

degradation product, cytochrome P-420, is inactive in mono-oxygenase reactions in mammalian systems. After an initial increase in P-420 concentrations during the first 3h of illumination this latter cytochrome also undergoes a biphasic period of decline in concentration, similar to that of the cytochrome P-450. This suggests that P-type cytochromes are degraded sequentially, perhaps with a common breakdown mechanism. The lability of the haem prosthetic group and accumulation of cytochrome P-450 apoprotein is well documented in porphyrinogenic rat livers (De Matteis, 1970). In plants the short-lived appearance and rapid disappearance of the P-type cytochrome may also be by specific attack on the haem prosthetic group.

In contrast, when light-grown seedlings with low concentrations of cytochrome P-450 are transferred to the dark, the net decrease ceases and a slow resynthesis takes place with a doubling time of some 30–40h. Unexpectedly, during dark-mediated net synthesis of cytochrome P-450, there is a slow build-up of cytochrome P-420 as well. Such results suggest that even in periods of net synthesis, cytochrome P-450 is subject to turnover. The accumulation of cytochrome P-420 on prolonged dark growth (Hendry *et al.*, 1981) suggests that degradation of its haem prosthetic group is relatively slow under these conditions.

The stability of the microsomal b-type cytochromes, some 80% being cytochrome b_{559} , was a consistent feature. Transfer to light, or dark, had no significant effect on cytochrome b concentrations and indicate that changes in concentration of the microsomal cytochromes are largely confined to cytochromes P-450 and P-420.

No significant light- or dark-mediated changes in cytochrome P-450 concentrations in older seedlings (5–7 days) were observed. This may be because during this period the endogenous concentrations of cytochromes P-450 and P-420 are rapidly declining. Failure to induce resynthesis on darkening in older tissue may indicate the loss of ability to synthesize any cytochrome P-450.

Light-mediated decreases and dark-mediated increases in cytochrome P-450 concentrations have substantially different half-times (about 6h and 30h respectively). This light–dark decrease–increase pattern would, therefore, under natural diurnal rhythm produce a net cytochrome P-450 concentration decrease. The possibility remains that cytochrome P-450, in young seedlings, functions principally in heterotrophic metabolism before or in the absence of the opportunity to establish an autotrophic way of life, the latter depending on reception of light.

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