The cytochromes in microsomal fractions of germinating mung beans

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(Received 31 July 1980/Accepted 7 October 1980)

Detailed studies of microsomal cytochromes from mung-bean radicles showed the presence of cytochrome *P*-420, particularly in dark-grown seedlings, accompanied by smaller quantities of cytochrome *P*-450. Similar proportions of cytochrome *P*-420 to cytochrome *P*-450 were found spectrophotometrically *in vivo* with whole radicles and hypocotyls. Assayed *in vitro*, maximum concentrations of both cytochromes were attained after 4 days of growth, before undergoing rapid degradation. Illumination of seedlings stabilized cytochrome *P*-450 and decreased the amount of cytochrome *P*-420. Three *b* cytochromes were present in the microsomal fraction, namely cytochromes *b*-562.5 ($E_m + 105 \pm 23$ mV), *b*-560.5 ($E_m + 49 \pm 13$ mV) and *b*₅ ($E_m - 45 \pm 14$ mV), all at pH 7.0. Of the *b* cytochromes, cytochrome *b*₅ alone undergoes a rapid degradation after day 4. Changes in cytochrome *b* concentrations were confined to the microsomal fraction; mitochondrial *b* cytochrome concentrations were unaltered with age. Protohaem degradation (of exogenous methaemalbumin) was detected in microsomal fractions of mung beans. The rates of degradation were highest in extracts of young tissue and declined after day 4. The degradation mechanism and products did not resemble those of mammalian haem oxygenase.

Plant microsomal fractions have been previously isolated from different plant species and shown to contain a very variable cytochrome composition (see, e.g., Rich & Bendall, 1975). Cytochrome *P*-450 has sometimes been detected or reported to be absent, and up to two of five different *b* cytochromes have been distinguished by their absorbance maxima or oxidation–reduction mid-point potentials (Rich & Bendall, 1975; Rich *et al.*, 1975; Rich & Lamb, 1977; Ishimaru & Yamazaki, 1977; Salaun *et al.*, 1978). Little evidence has been published showing the development of microsomal cytochromes during plant growth, or on the effect of light and dark growth regimes. Indeed, such extraordinary variations have been reported (see, e.g., Rich & Bendall, 1975) in the concentration of microsomal cytochromes in different species that no serious attempt can be made to gauge the principal functions of such cytochromes, particularly cytochrome *P*-450, during plant development.

In light-grown pea seedlings, cytochrome *P*-450 concentrations show a linear increase during the first 5 days of growth when expressed per g dry wt. of seed (Ishimaru & Yamazaki, 1977). However, our own observations (G. A. F. Hendry & O. T. G. Jones, unpublished work) showed that this pattern does not hold for germinating soya beans and barley, nor for tulip and daffodil bulbs during

dormancy break. In contrast, in the mammalian hepatic microsomal system, cytochrome *P*-450 concentrations increase, more or less steadily, with body weight from embryo to mature animal (Siekevitz, 1965), though absolute concentrations vary considerably in different organs and species (Oshino, 1978).

In plants, part of the microsomal electron-transport system has been implicated in the hydroxylation of phenolic, terpenoid and fatty acid substrates (Murphy & West, 1969; Meehan & Coscia, 1973; Abdelkader *et al.*, 1973; Rowell *et al.*, 1974; Ishimaru & Yamazaki, 1977; Rich & Lamb, 1977), reactions considered to be analogous to those catalysed by mammalian *P*-450 cytochromes (Ishimaru & Yamazaki, 1977). However, the function of the plant system is likely to differ from that of the well-characterized liver system, if only because the latter is functionally linked to detoxification and excretory processes (De Matteis, 1981). We confirm, however, that microsomal fractions from mung beans and other plant species (Hendry & Jones, 1978) do appear to have regulatory properties over haem metabolism, as shown by an age-linked capacity for haem degradation and cytochrome disappearance. Such properties are comparable with the mammalian hepatic haem oxygenase system.

In view of the dearth of information on the

function and developmental aspects of plant microsomal cytochromes, we have undertaken a systematic study of the haem protein content of germinating mung beans with a view to establishing whether the reported differences in plant cytochrome content can be explained from alterations during development and by the effect of light or dark growth. We have found that both age and illumination regimes have a profound effect on the haemo-protein content of the microsomal fractions.

Materials and methods

Materials

Mung-bean (*Phaseolus aureus*) seeds were purchased locally. Analytical-grade chemicals were supplied by BDH Chemicals, Poole, Dorset, U.K.; horse heart cytochrome *c* and NADH (grade II) were from Boehringer, Mannheim, Germany; bovine haemin, bovine liver catalase and bovine blood superoxide dismutase (type 1) were from Sigma (London) Chemical Co., Poole, Dorset, U.K.

Isolation of microsomal fraction and mitochondria

Mung beans were imbibed for 16 h in water, sown on dampened paper towels and grown in darkness or light (from 60 W tungsten bulbs, 0.5 m above seedling level) at 20–23°C. At intervals, the seedlings (120–240 g fresh wt.) were harvested and homogenized (Ato-Mix blender, 5 s) in 0.25 M-sucrose/10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid/10 mM-Tricine {*N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine}/0.1% bovine serum albumin/0.1 mM-dithioerythritol buffer, pH 7.6. The homogenate was squeezed through three layers of Miracloth supported on muslin, and the pH was re-adjusted to 7.6 before centrifugation at 20 000 *g* for 30 min. The resulting supernatant was re-centrifuged at 100 000 *g* for 3 h and the microsomal pellet was resuspended in extraction medium (minus dithioerythritol). For preparations of mitochondria, the 20 000 *g* pellet was resuspended, the starch and plastid fractions were removed by centrifugation at 4000 *g* for 15 min and the mitochondria were pelleted by centrifugation at 12 000 *g* for 20 min before final resuspension in extraction medium.

Cytochrome assays

Mitochondrial and microsomal cytochromes were assayed by their dithionite-reduced-minus-aerated difference spectra (cytochrome *a*, $A_{605}-A_{630}$, $\epsilon = 16 \text{ mM}^{-1} \cdot \text{cm}^{-1}$; cytochrome *b*, $A_{559}-A_{570}$, $\epsilon = 20 \text{ mM}^{-1} \cdot \text{cm}^{-1}$; cytochrome *c*, $A_{550}-A_{540}$, $\epsilon = 20 \text{ mM}^{-1} \cdot \text{cm}^{-1}$), cytochromes *P*-450 and *P*-420 by their reduced + CO-minus-reduced difference spectra (cytochrome *P*-450, $A_{452}-A_{490}$, $\epsilon = 91 \text{ mM}^{-1} \cdot \text{cm}^{-1}$; cytochrome *P*-420, $A_{421}-A_{490}$,

$\epsilon = 110 \text{ mM}^{-1} \cdot \text{cm}^{-1}$, further adjusted for the cytochrome *P*-450 trough at 420 nm by the method of Estabrook *et al.*, 1972). Cytochrome *P*-450 was also assayed by its subsequent conversion into cytochrome *P*-420 by the addition of KSCN (final concn. 20 mM, incubation time 10 min).

Spectra of cytochromes *in vivo* were recorded by using a 3 mm-path-length cuvette packed with radicles and incorporating a 1 mm (internal diam.) micro-bore glass tube as an entry port for Na₂S₂O₄ (50 mM), KSCN (about 20 mM) and CO, without the need to disturb the plant tissue arrangement.

Protohaem isolation from haem proteins, by using HCl/acetone and assay by pyridine haemochromogen formation, followed the method of Castelfranco & Jones (1975). The cytochromes *c* in the residue were also assayed as the pyridine haemochromogen.

Oxidation–reduction potentiometry

Oxidation–reduction potentiometric titrations were performed by using the method of Dutton (1978) in a stirred cuvette fitted with platinum and calomel electrodes under an argon atmosphere. Because the presence of contaminating carotenoids caused base-line shifts, a split-beam scanning spectrophotometer was used in preference to a double-beam spectrophotometer. To prevent microsomal precipitation by high salt concentrations during potentiometry, the particles were resuspended in 0.25 M-sucrose/10 mM-KCl/20 mM-Mops (4-morpholinepropanesulphonic acid) buffer, pH 7.0. The mediators added to the suspension were: phenazine methosulphate (25 μM), phenazine ethosulphate (25 μM), pyocyanine (6 μM), 3,6-diaminodurene (25 μM), 2-hydroxy-1,4-naphthoquinone (20 μM), duroquinone (25 μM), quinol (hydroquinone) (25 μM), anthroquinone (10 μl of ethanol-saturated solution) and anthroquinone-2,6-disulphonate (25 μM). The range of potentials investigated was from +300 mV to –400 mV. Redox titration data were simultaneously computer-stored to provide spectra at various potentials.

Enzyme assay

Antimycin A-insensitive NADH–cytochrome *c* reductase activity was measured by the method of Martin & Morton (1956).

Haem degradation assays

Methaemalbumin was prepared from bovine haemin and bovine serum albumin (otherwise by the method of Tenhunen *et al.*, 1968). A 1.5 nmol portion of methaemalbumin was incubated with a microsomal fraction (10–15 mg of protein/ml) suspended in extraction medium (minus dithioerythritol) at pH 7.4. At timed intervals samples were withdrawn and diluted 8-fold in alkaline pyridine

(0.3 M-KOH/pyridine, 3:2, v/v). The solution was divided between two cuvettes, and the sample cuvette was reduced with a few crystals of solid $\text{Na}_2\text{S}_2\text{O}_4$. At 90 s after pyridine dilution, the haemochromogen difference spectrum was recorded; the extent of haem breakdown was measured by the decrease in absorbance at 557–540 nm in successive samples. pH optima were measured by using 10 mM-Mes (4-morpholine-ethanesulphonic acid)/Mops or -Tricine buffers. Anaerobic incubations were prepared in Thunberg tubes evacuated and flushed with N_2 . For assay for the presence of biliverdin α -IX, biliverdin reductase was prepared from 100 000 g (1 h) supernatants from perfused rat livers prepared as previously described (Lodola *et al.*, 1979).

Protein assays

Proteins were assayed by the method of Bramhall *et al.* (1969).

Results

Growth and developmental characteristics of mung beans

Under dark or light growth conditions, radicles of germinating mung beans undergo a linear increase in length for the first 4 days; thereafter growth slows with the development of lateral roots. Examination of the cell debris, after the blending procedure, showed that the bulk of the crude filtrate, used subsequently in preparation of the microsomal fraction, was derived from radicle, root and hypocotyl tissue, not cotyledons or leaves, which were resistant to homogenization.

Protein concentrations per g fresh wt. decline rapidly with age, both in total crude homogenates and in microsomal pellets. For the latter, microsomal protein per g fresh wt. declined from 1.5 mg at day 1 to 0.5 mg by day 5 and to 0.3 mg by day 8.

Mung-bean microsomal preparations contain considerable quantities of carotenoids, a feature common to other plant species. Additional purification of the microsomal fraction by sucrose-density-gradient centrifugation did not significantly remove these pigments. In light-grown preparations, particularly 7–8-day-old material, small amounts of chlorophylls contaminated the microsomal fraction. These pigments make cytochrome absorption difference spectra base-line corrections difficult to resolve, a problem alluded to by others workers in similar studies (Rich, 1975; Rich & Lamb, 1977). In the present work satisfactory resolution was obtained only by repetitive spectral scanning to achieve relatively straight base-lines.

Cytochromes P-450 and P-420

Spectra of microsomal cytochromes from 3-

day-old dark-grown mung beans are shown in Fig. 1. There was a predominance of cytochrome P-420 over cytochrome P-450. Although the former is considered as a degradation product of the latter, at least in mammalian systems, it is possible that other microsomal haem proteins may form similar CO complexes absorbing at 420 nm. Alterations to the dithioerythritol concentrations in the extraction medium, dithioerythritol addition to the suspension medium or the use of dithiothreitol or cysteine (to 0.2 mM) in an attempt to prevent cytochrome P-420 formation failed. To investigate whether the large amounts of cytochrome P-420 were an artifact of extraction, spectra of mung-bean radicles and hypocotyls *in vivo* were determined. These consistently gave cytochrome P-420/cytochrome P-450 ratios similar to those found *in vitro*, namely between 6:1 and 3:1 (Fig. 1). The only marked difference between spectra obtained *in vitro* and *in vivo* was the shift of the cytochrome P-450 absorbance maximum from 452 nm to 454–456 nm *in vivo*. Additions of KSCN (20 mM) to whole roots after CO binding resulted in the conversion of cytochrome P-450 into the 422 nm-absorbing

Fig. 1. Spectra of microsomal fraction from 3-day-old dark-grown mung beans

Experimental details are as given in the text. Against a base-line (---) is shown the dithionite-reduced-minus-aerated difference spectrum (—). Above (····) is the dithionite-reduced + CO-minus-dithionite-reduced difference spectrum of cytochromes P-420 and P-450. Inset shows a similar dithionite-reduced + CO - minus - dithionite-reduced difference spectrum of whole radicles (see the text for details).

species, confirming the identity of the cytochrome *P*-450. It appears that in mung beans cytochrome *P*-420 is not an artifact of extraction but may arise *in vivo*, perhaps from degradation of cytochrome *P*-450.

Concentrations of cytochromes *P*-450 and *P*-420 change with age and with light and dark treatments. Fig. 2 shows that cytochrome *P*-450 concentrations rise sharply in etiolated seedlings, reaching a maximum concentration of 0.066 nmol/mg of microsomal protein at day 4, declining rapidly in the succeeding days. Light-grown tissue, however, has considerably lower concentrations of cytochrome *P*-450, i.e. about 0.025 nmol/mg of microsomal protein during the period from 2 to 4 days with a rapid disappearance by day 6. A similar pattern was observed with cytochrome *P*-420; maximum concentrations (0.30 nmol/mg of protein) were found in dark-grown tissue at day 4 compared with 0.06 nmol/mg of protein in illuminated preparations.

The cytochrome *P*-450/cytochrome *P*-420 ratios differ significantly in light and dark growth, and show (Fig. 3) that, despite the lower concentrations of cytochrome *P*-450, cytochrome *P*-450/cyto-

chrome *P*-420 ratios were constantly higher in light-grown plants. Approx. 35–40% of the total cytochrome *P*-450 plus cytochrome *P*-420 consists of cytochrome *P*-450 in illuminated tissue compared with 15–20% in dark-grown seedlings.

NADH–cytochrome *c* reductase (antimycin-insensitive), used as a marker of microsomal activity, showed a developmental pattern similar to that of cytochrome *P*-450, reaching maximum activity at day 5 of 34 and 26 nmol/min per mg of microsomal protein (light and dark treatments respectively). Reductase activity declined rapidly thereafter.

Other cytochromes

Mung-bean microsomal fractions are reported to contain cytochrome *b*₅ (Shichi & Hackett, 1962), with a split α -band (λ_{max} 555 and 559 nm) but with no published mid-point potential, and a cytochrome *b*-562 with a mid-point potential of $+120 \pm 20$ mV (Rich & Bendall, 1975), the latter apparently being the major component in the etiolated mung-bean microsomal fraction studied by Rich & Bendall (1975).

Difference spectra of microsomal fractions from 3–5-day-old tissue showed an absorbance maximum at 559 nm with a distinct shoulder at 555 nm. By day 7–8 there was an apparent shift in the absorbance maximum from 559 nm towards 560–561 nm. Changes with age in the total cytochrome *b* concentrations (measured at the absorbance maxima) in the light and dark treatments are shown in Fig. 4. Under both growth conditions maximum concentrations are reached by day 5, and are 2.0 and 1.24 nmol/mg of microsomal protein in the light and dark respectively. Unlike cytochromes *P*-450 and *P*-420, *b* cytochromes are present in greater amounts in illuminated tissue than in etiolated seedlings. As with cytochromes *P*-450 and *P*-420, however, there is a sharp decrease in cytochrome *b* concentrations after day 5.

Fig. 2. Changes in the concentrations of cytochrome *P*-420 (a) and *P*-450 (b) with age in microsomal fractions from dark-grown (●) and light-grown (○) mung beans. Experimental details are given in the text. Determination of concentrations was from dithionite-reduced + CO-minus-dithionite-reduced difference spectra. Additions of KSCN (see the Materials and methods section) was used to convert cytochrome *P*-450 into cytochrome *P*-420 as an alternative means of quantifying low concentrations of cytochrome *P*-450.

Fig. 3. Plots of cytochrome *P*-450 concentration against cytochrome *P*-420 concentration in light-grown (○) and dark-grown (●) mung-bean microsomal fractions. The data were derived from the spectra shown in Fig. 2 from material 1 to 8 days old.

Fig. 4. Concentration of total *b*-type cytochromes, with age, in microsomal fractions from dark-grown (●) and light-grown (○) mung beans

Experimental details are given in the text. The values were recorded at the α -band absorbance maxima initially at 559 nm, shifting with age to 560–561 nm.

The microsomal content of cytochromes *a* plus *a*₃ and cytochrome *c* was examined in spectra at room temperature and 77K to determine the extent of mitochondrial contamination. Cytochrome oxidase contamination was less than 7% of the total microsomal cytochromes at day 1, decreasing to undetectable amounts by day 4 and thereafter. After acid/acetone extraction, pyridine haemochromogen analysis of microsomal fractions showed only trace (<1%) contamination by cytochrome *c*. In spectra at 77K it was undetectable at all ages.

At least four different plant microsomal cytochrome *b* species have been reported in the literature (Rich & Bendall, 1975), namely 'classical' cytochrome *b*₅ and cytochromes *b*-561, *b*-562 and *b*-559.5. In our studies three distinct cytochromes were found in microsomal fractions from mung-bean seedlings of all ages, comprising cytochrome *b*₅, with characteristic split absorption maxima (554 and 558 nm at 77K), and two other components, with room-temperature absorption maxima at 560.5 nm and 562.5 nm respectively as derived from oxidation–reduction potentiometry (see below). The very-low-potential component described by Rich & Bendall (1975) was also present, but as a minor component in our experiments.

Oxidation–reduction potentiometry

Oxidation–reduction mid-point potentials of microsomal cytochromes from 11 different seedling

Fig. 5. Redox-potential titrations of *b*-type cytochrome components from the microsomal fraction of 4-day-old dark-grown mung beans

Experimental details are given in the text. The data show the mid-point potentials of cytochrome *b*562.5 (●) (E_m +118 mV), cytochrome *b*-560.5 (▲) (E_m +48 mV) and cytochrome *b*₅ (■) (E_m –47 mV).

ages and light/dark growth conditions were calculated (see Fig. 5). The mid-point potentials were: cytochrome *b*-562.5, +105 ± 22.6 mV; cytochrome *b*-560.5, +49 ± 12.9 mV; cytochrome *b*₅, –45 ± 13.9 mV. The cytochrome *b*₅, when measured at 555 nm instead of 559 nm (isosbestic point 570 nm), showed an identical mid-point potential of –45 ± 14.2 mV. Variations within the samples are given as standard deviations.

Computer storage of the redox titration data allowed difference spectra to be recorded at selected potentials, and, by subtracting higher-potential components, spectra of cytochromes at given potentials were obtained. Fig. 6 shows the α - and β -bands of four components, A being a very-low-potential component previously referred to by Rich & Bendall (1975). In our titrations the spectrum of component A was obtained by using catalytic amounts of Benzyl Viologen and Methyl Viologen as additional mediators; the spectrum represents the difference between the reference cuvette (E_h –320 mV) and the

Fig. 6. Spectra of α - and β -bands of four redox-potential components from the microsomal fraction of 4-day-old dark-grown mung beans

Experimental details are given in the text. The spectra coincide approximately with the mid-point potentials established for each component. In every case the spectral contribution of the higher-potential component(s) was subtracted from the next (lower-) potential component. Component B corresponds to cytochrome b_5 , component C to cytochrome b -560.5, and component D to cytochrome b -562.5. The component A (see the text) corresponds in mid-point potential to cytochrome P -450 but exceeds in amount the known concentration of cytochrome P -450.

dithionite-reduced-sample cuvette (estimated $E_h > -600$ mV). The approximate mid-point potential of component A was about -300 to -330 mV, close to that reported for cytochrome P -450 (Rich *et al.*, 1975), though the concentration (assuming $\epsilon = 20 \text{ mM}^{-1} \cdot \text{cm}^{-1}$) was severalfold greater than that calculated from the sum of cytochrome P -450 plus cytochrome P -420 CO-binding spectra, and makes this low-potential compound A unlikely to be cytochrome P -450. The spectrum of component B is a room-temperature spectrum for cytochrome b_5 obtained by subtracting all components above 0 mV, the spectrum of component C is that of cytochrome b -560.5 calculated by subtracting the spectrum of the component above $+70$ mV, and the spectrum of component D is that obtained at $+100$ mV. As there was a considerable decrease in microsomal b cytochromes with age, an attempt was made to identify the cytochrome species subject to age-linked degradation. By using the spectra obtained during

oxidation–reduction potentiometry, it was possible to quantify the contribution made by cytochrome b_5 in relation to the higher-potential components by taking $+10$ mV as a convenient titration end point. Although the oxidized end point of cytochrome b_5 overlaps with the reduced end point of cytochrome b -560.5, at 10 mV cytochrome b_5 would be largely (approx. 90%) in the oxidized state and the two higher-potential cytochromes totally or substantially in the reduced state. Ratios of cytochrome b_5 to the total higher-potential components were thus calculated. Fig. 7 shows that, independently of illumination during growth, during days 3 and 4 the ratio of low-potential to high-potential cytochromes was about 5.5:1, declining by day 5 to 3.5:1, and by day 7 and 8 falling to 1:1 or less. The change in ratios was due substantially to a major loss of cytochrome b_5 (between day 4 and 5 from 1.48 to 1.02 nmol/mg of protein), with small increases in high-potential components (0.26 to 0.35 nmol/mg of protein in the same period). This loss of cytochrome b_5 , both in absolute and relative amounts, coincides with the loss of cytochrome P -450 (and cytochrome P -420) and in NADH–cytochrome c reductase activity with age.

As the concentration of microsomal cytochromes undergoes considerable change with age, a comparative study was made of mitochondrial cytochromes from the same mung-bean preparations. Table 1 shows that the total mitochondrial cytochrome oxidase per seed steadily increases in amount throughout the growth period. Mitochondrial cytochromes b and c increase more rapidly to day 3, but show little change thereafter. In contrast, the microsomal cytochromes undergo considerable changes in concentration over this time course. No cytochrome P -450 or cytochrome P -420 was detected in mitochondrial preparations.

Haem turnover

Since our results suggested that microsomal haemoproteins were degraded during growth, an assay of the degradation of protohaem (the haem prosthetic group of cytochromes P -450 and P -420 and of b -type cytochromes) was devised. The characteristics of this degradation mechanism have been previously briefly outlined (Hendry & Jones, 1978), and indicate that protohaem (supplied as methaemalbumin) was broken down by mung-bean microsomal fraction in a reaction that has an absolute requirement for molecular O_2 (anaerobic incubations showing zero activity). Neither added catalase (0.1 kat) nor superoxide dismutase (0.15 kat) had any significant effect on haem breakdown rates. The microsomal degradation activity was destroyed by heating at 100°C (81% in 30 s), and the pH optimum (in the range pH 6.1–8.5) was 7.4. The system, unlike the mammalian haem

Fig. 7. Ratio of low-potential (<10 mV) to high-potential (>10 mV) components in microsomal fractions from mung beans grown in the dark (●) or light (○) at different ages
The data were derived from spectra obtained during redox potentiometry by comparing the absorbance of components reduced over the range +150 to +10 mV with that reduced at between +10 mV and –200 mV.

Table 1. Concentrations of cytochromes from mung-bean microsomal fraction and mitochondria at various ages
Experimental details are given in the text. The values for both mitochondrial and microsomal fractions were derived, at each age, from the same plant tissue and show the apparent stability of mitochondrial cytochromes against the age-linked degradation of b-type cytochromes in the microsomal fraction.

Age (days)	Cytochrome content (nmol/100 g dry wt. of seeds)			
	Mitochondrial fraction			Microsomal fraction
	Cytochromes a + a ₃	Cytochrome b	Cytochrome c	Cytochrome b
2	7.2	14.9	12.7	24.8
4	13.8	44.4	25.0	22.4
6	13.7	64.4	33.7	13.3
8	19.4	51.3	33.6	10.8

oxygenase reaction, does not require an exogenous cofactor. Neither protoporphyrin IX nor a bile pigment was detected as a product of degradation, nor did the product react with mammalian biliverdin reductase. The rates of haem breakdown activity declined from 0.71 (day 3) to 0.14 (day 5) to 0.12 nmol/h per mg of microsomal protein (day 7) in light-grown samples.

Discussion

The concentration of mung-bean cytochrome

P-450, at its optimum of 0.066 nmol/mg of microsomal protein (day 4), is similar to that of germinating pea cotyledons and cauliflower buds (Rich & Bendall, 1975). In mammals cytochrome P-450 concentrations typically range from about 0.03 nmol/mg of protein in rat brain (Holtzman & Desautel, 1980) to over 1.0 nmol/mg of protein in rat liver (Matsubara *et al.*, 1976) or more in other species (Omura & Sato, 1964).

Cytochrome P-420 concentrations in mung beans and other plant species (Rich & Bendall, 1975) appear to be considerably higher than in mammals.

In our studies the relatively high concentrations of cytochrome *P*-420, compared with cytochrome *P*-450, both *in vivo* and *in vitro* suggest that the turnover of the former is comparatively slow if cytochrome *P*-420 is the natural degradation product of cytochrome *P*-450 (see De Matteis, 1981). Although the absolute concentration of cytochrome *P*-450 was substantially lower in illuminated tissue than in etiolated material, the concentration of cytochrome *P*-450 relative to that of cytochrome *P*-420 was consistently higher in light-grown seedlings, owing perhaps to a light-induced stabilizing environment for cytochrome *P*-450.

Three *b*-type cytochromes were found in mung-bean microsomal fractions. The cytochromes *b*-560.5 and *b*-562.5 do not correspond in mid-point potentials to the *b*-type cytochromes from chloroplasts (Rich & Bendall, 1980) nor to mitochondrial or plastid contamination. They may be, however, comparable with one or more of the various microsomal cytochromes reported for other plant species (Rich & Bendall, 1975). The major *b*-type cytochrome in mung-bean microsomal fractions is cytochrome *b*₅, at least during the first 6 days of growth. The overall decrease in *b*-type cytochromes after day 5 appears to be confined to cytochrome *b*₅, both in light-grown and in dark-grown tissue, and is unlikely to be due to the small growth differences between radicle and hypocotyl. The ratios of *b*-type cytochromes to cytochromes *P*-450 plus *P*-420 vary with age from 10:1 to 50:1 (3-day-old and 5-day-old light-grown) and 2:1 to 6:1 (3-day-old and 5-day-old dark-grown), values within the wide range reported for other plant species (Rich & Bendall, 1975). In contrast, cytochrome *b*₅/cytochrome *P*-450 ratios in mammals are usually in the region of 1:1 to 1:2 (rabbit and rat liver microsomal fractions) (Omura & Sato, 1964; Matsubara *et al.*, 1976). Only in rat brain microsomal fractions are high cytochrome *b*₅/cytochrome *P*-450 ratios (about 3:1) reported (Holtzman & Desautel, 1980). Oshino (1978) considered that cytochromes *b*₅ and *P*-450 occur together in 'mixed-function oxidase' complexes in most mammalian organs, with the exception of brain tissue, where he considered that the excess of cytochrome *b*₅ over cytochrome *P*-450 indicated that cytochrome *b*₅ may exist as an independent reductase. We conclude that in mung beans the large excess of *b*-type cytochromes (with cytochrome *b*₅ as the major component) over cytochromes *P*-450 plus *P*-420 makes it unlikely that plant microsomal *b*-type cytochromes are necessarily functionally linked to cytochrome *P*-450. This is particularly so in 6–8-day-old seedlings, where cytochrome *b*₅ is present but cytochrome *P*-450 is undetectable. Similarly it is difficult to reconcile the extreme variations in the *b*-type cytochromes/cytochromes *P*-450 plus *P*-420 ratios

of 50:1 and 6:1 in 5-day-old light-grown and dark-grown seedlings. The excess of cytochrome *b*₅ in plants implies an additional function (other than cytochrome *P*-450 reduction), or indeed a loss of function with development, for this cytochrome.

The rapid net degradation of cytochromes, particularly the *b*-type cytochromes, becomes apparent with age, but only in the microsomal fraction and not in mitochondria. Mung-bean microsomal fraction contains a system for the breakdown of protohaem (the prosthetic group of *b*-type cytochromes and cytochrome *P*-450) *in vitro*. The mechanism differs in several respects from the mammalian microsomal haem oxygenase system. In plants, the haem-degrading mechanism appears to be involved in the removal of microsomal cytochromes at critical stages of development. This mechanism may also function in the overall regulation of plant porphyrin metabolism via control of 5-aminolaevulinic acid synthesis (Hendry & Stobart, 1977, 1978), as in the mammalian and bacterial systems (Granick & Beale, 1978).

The functions of cytochromes *P*-450 and *b*₅ in plant microsomal fractions appear to correspond to certain of those in mammalian systems (e.g. hydroxylation of lipid-soluble compounds), though the overall role of cytochrome *b*₅ remains elusive both in mammals (Oshino, 1978; Holtzman & Desautel, 1980) and in plants (Rich & Lamb, 1977). However, our evidence suggests that there exists, in plants, a short-lived requirement for cytochrome *P*-450-mediated reactions that may become largely redundant as the seedling achieves photosynthetic competence or, in etiolated tissue, approaches premature senescence.

G. A. F. H. gratefully acknowledges the financial support of the Medical Research Council.

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