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PHYTOCHROME-MEDIATED PLASTID DEVELOPMENT IN ETIOLATED PEA STEM APICES

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Abstract—The effects of red and far-red light on growth and plastid development in the stem apices of etiolated pea seedlings have been examined. Changes were determined in various growth parameters (DNA, soluble protein and fresh weight) and also in the activities of the plastid-localized enzymes ribulose-1,5-bisphosphate carboxylase, NADP-glyceraldehyde-3-phosphate dehydrogenase and alkaline-1,6-bisphosphatase and the non-photosynthetic (cytoplasmic) enzymes NADP-isocitrate dehydrogenase, enolase and NAD-malate dehydrogenase. Changes in the amounts of Fraction I protein were also measured. Brief daily irradiation with low intensity red light increased growth 5·1–7·6-fold which was correlated with increases of about 3·5-fold in activities of the non-photosynthetic enzymes. The chloroplast enzymes, however, showed much greater increases in activity ranging from 15- to 91-fold. Fraction I protein increased 11·7-fold. These increases approached the levels attained in fully green leaves. All these responses were largely prevented by far-red light indicating that they were mediated by phytochrome. In experiments with red light given at daily intervals there was a lag of 24 hr before the initially very low activity of ribulose-1,5-bisphosphate carboxylase increased. Fraction I protein which was initially present in significant amounts showed a similar lag in its synthesis. However, for 3 days after the initial irradiation, the rate of increase of the enzymic activity was much greater than the rate of net synthesis of Fraction I protein. A single initial red irradiation was as effective as 3 daily irradiations in increasing the activity of ribulose-1,5-bisphosphate carboxylase. A fourth irradiation, however, gave an additional response which exceeded that of the single initial irradiation. It was shown that there was a rapid activation of ribulose-1,5-bisphosphate carboxylase by either continuous white or 3 min of red light. The red light response was slowly reversed in the dark. These results are discussed with particular emphasis on the relation between growth and plastid development in a phytochrome-mediated system.

INTRODUCTION

PHYTOCHROME mediates a wide variety of morphogenetic responses in plant tissues. Most of the parameters measured have been major growth responses and until recently little information on the regulation of enzyme synthesis mediated by phytochrome has been available. It has been shown[1] that etiolated bean seedlings synthesized increased amounts of protein and lipids in the dark following brief exposure to low intensity red irradiation (R). Thus R has been shown to increase the activity of phenylalanine ammonia-lyase (E.C. 4.3.1.5) in mustard seedlings (*Sinapis alba*)[2] and a chloroplast localized enzyme, NADP-glyceraldehyde-3-phosphate (G-3-P) dehydrogenase (E.C. 1.2.1.13) in bean.[3] Irradiation with R followed by far-red light (FR) resulted in inhibition of increases in protein, lipid and the activities of enzymes. The mediation of phytochrome in the increases can be inferred from these findings.

In etiolated rye seedlings, light-induced increases in activities of ribulose-1,5-bisphosphate (RuP) carboxylase (E.C. 4.1.1.39) transketolase (E.C. 2.2.1.1) and, probably, ribose-

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phosphate isomerase (E.C. 5.3.1.6) were shown to be mediated by phytochrome.[4] These increases in enzymic activities were not more than three-fold over the dark controls.

In a previous paper[5] we have shown that for etiolated pea seedlings, increases in activity of RuP carboxylase per stem apex were about 90-fold above the dark control following brief, daily irradiations with low intensity R (λ_{max} 661 nm). Large increases were also found in NADP-G-3-P dehydrogenase (35-fold) and alkaline fructose-1,6-bisphosphatase (E.C. 3.1.3.11) (15-fold). FR following the R resulted in much lower increases, implicating phytochrome in these responses. Further, the increases induced by R were much greater than would have been expected from the growth responses induced simultaneously. The increases in RuP carboxylase activity were paralleled, at least in the longer term treatments, by increases in Fraction I protein[5] with which this enzyme is associated.[6–10] Furthermore, the increases in activity of RuP carboxylase were a substantial fraction of the level attained in fully green pea leaves.

Phytochrome-mediated increases have been reported[11] in the activities of several photosynthetic and non-photosynthetic enzymes after brief illumination of etiolated bean seedlings with white light. The increases were from 1·5- to 6·3-fold while growth, measured on a fresh weight or protein basis, was stimulated about 2-fold.

The magnitude of the increases obtained with etiolated rye[4] and bean[11] seedlings are much smaller than those obtained with etiolated pea.[5] In order to explain this disparity, further experiments on pea and a different cereal, wheat, are reported in this and a following paper. Particular regard is paid to the relation between growth and RuP carboxylase activity and the amount of Fraction I protein. Results are reported for some other photosynthetic enzymes and for some non-photosynthetic enzymes which might be expected to correlate with growth responses. The possible photo-activation of RuP carboxylase is also described.

RESULTS

Growth of Pea Stem Apices following Brief Irradiation with Red Light

Patterns of growth have a significant bearing on the interpretation of the changes in enzymic activities which irradiation treatments induce in etiolated pea stem apices. Accordingly, particular attention was paid to changes in amounts of DNA, soluble proteins and fresh weight following various irradiation treatments.

Figure 1 shows the combined results of three experiments on 7-day-old, etiolated pea seedlings to determine the effects of R on growth as measured by DNA content of the stem apices. Controls kept in total darkness were included and in one experiment comparisons were made with seedlings which were allowed to turn green in either continuous or intermittent (day/night) white light. A short, 3 or 5 min, period of R (72 μW/cm^2; 662 nm) was given daily to plants otherwise kept in the dark. Since 5 min of red light was no

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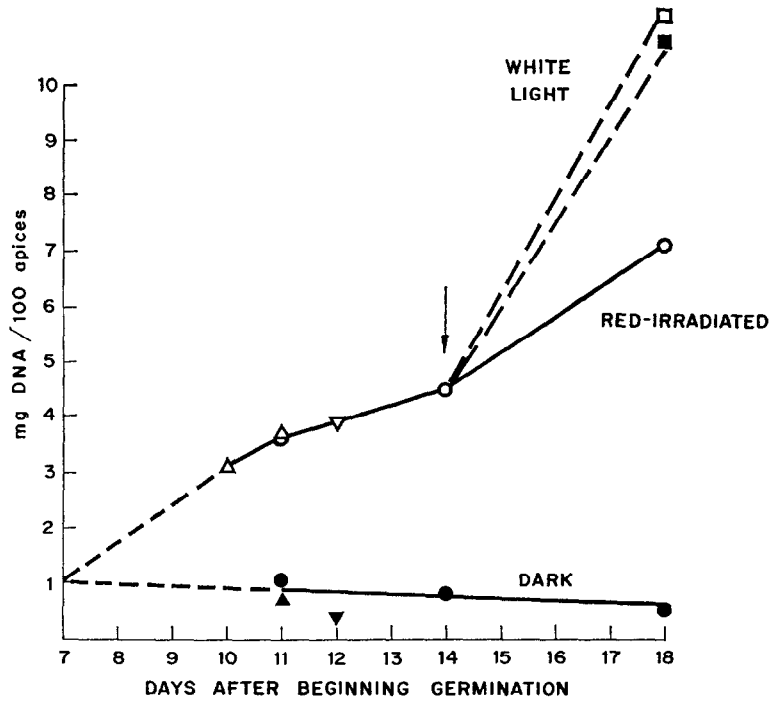

FIG. 1. EFFECT OF IRRADIATION WITH SHORT PERIODS OF RED LIGHT UPON DNA LEVELS IN STEM APICES OF ETIOLATED PEA SEEDLINGS.

Seven-day-old dark-grown seedlings were irradiated with R, and daily on subsequent days, for 10 min △ (Expt. 1.), 5 min, ▽ (Expt. 2.) and 3 min, ○, (Expt. 3) and returned to darkness. ▲, ▼ and ● represent the controls maintained in total darkness. In Expt. 3 some batches of seedlings after 7 daily red irradiations, that is, on the 14th day after the beginning of germination, were transferred to white fluorescent light given either continuously, □, or intermittently (16 hr light/8 hr dark), ■. The transfer to white light is indicated by the arrow.

more effective than 3 min in inducing the measured responses, the shorter period was apparently saturating.

The results show that, compared with the dark controls which changed little with time, R induces a large increase in DNA per apex suggesting that considerable cell division occurs. These plants remained etiolated and accumulated only 10 μg of chlorophyll a per g fresh weight compared with about 500 μg/g fresh weight in green tissue.

The red-irradiated plants which were transferred to white light became fully green in 4 days and showed further increases in DNA per apex of about 55 per cent compared with plants of the same age but irradiated daily with a short period of R only. These plants, therefore, contained about two-thirds of the amount of DNA found in the green plant.

Figure 2 gives the combined results of two experiments in which the two growth parameters, soluble protein and fresh weight were measured. The results are expressed per unit DNA. R induces an initial decrease in both growth parameters per unit DNA. Since the amount of DNA per apex increased about 3-fold in the 10-day-old red-irradiated seedling apices compared with the dark-grown controls (Fig. 1) it is apparent that the major initial response to R is one of cell division. This is followed by an increase in fresh

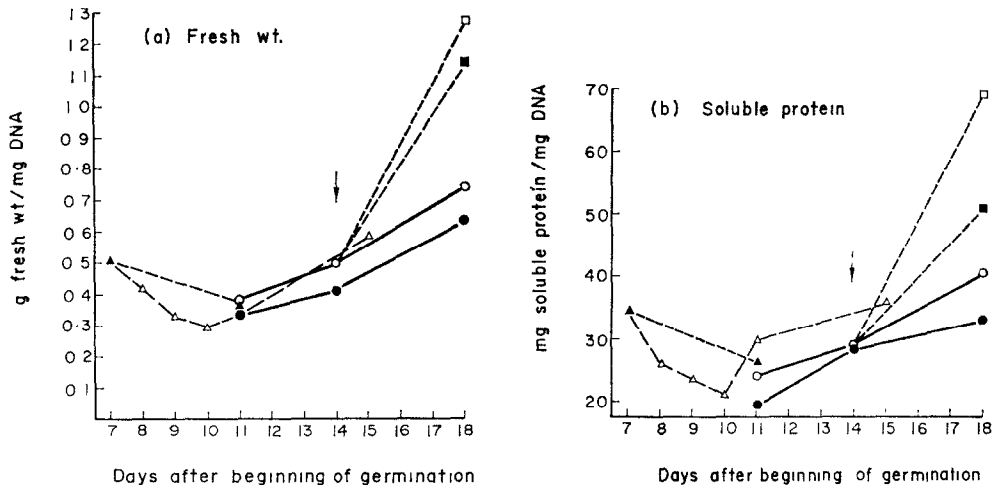

FIG. 2. EFFECTS OF IRRADIATION WITH RED LIGHT UPON A, FRESH WEIGHT PER UNIT DNA AND B, SOLUBLE PROTEIN PER UNIT DNA IN STEM APICES OF ETIOLATED PEA SEEDLINGS

Symbols refer to the same experiments as in Fig. 1. Arrows indicate transfer to white light.

weight and soluble protein per unit DNA. Since the amount of DNA per apex and presumably, therefore, cell number, is also increasing rapidly during this later period (Fig. 1), red irradiation results in substantial growth of the apices. White illumination after 7 daily red irradiations results in further growth, measured by the increases in soluble protein and fresh weight, over and above that resulting from R alone.

Changes in RuP Carboxylase and Fraction I Protein following Red Irradiation

Multiple irradiations. Figure 3 shows the effects of red light on RuP carboxylase activity and amount of Fraction I protein. The variation in enzymic activity per mg DNA (Fig. 3a) or per mg soluble protein (Fig. 3b) are similar. There is an initial lag in the increase in activity, only a very small increase being evident 24 hr after the first irradiation. Subsequently, however, a steady increase in activity occurs.

Fraction I protein with which RuP carboxylase activity is associated shows, on a per unit DNA basis, a slight fall in the first hr after the initial red irradiation There is then no substantial change until between the 10th and 11th day (Fig. 3c). Synthesis of Fraction I protein thus keeps pace approximately with DNA synthesis. After the 10th day there is an increase in Fraction I protein per unit DNA. Soluble protein per unit DNA also increases during this time (Fig. 2b) but the increase in Fraction I protein exceeds the increase in total protein (Fig. 3d).

Single initial irradiation. The results involving multiple irradiations may be compared with those in which only a single initial red irradiation was given to 7-day-old plants. Table 1 summarizes the effects on various growth parameters. Comparison with results in Fig. 1 shows that a single irradiation results in the synthesis of less DNA per apex. The changes in soluble protein and fresh weight are similar, on a DNA basis, to those of plants given multiple irradiations (see Fig. 2) so that these changes appear to be related to the rate of cell division.

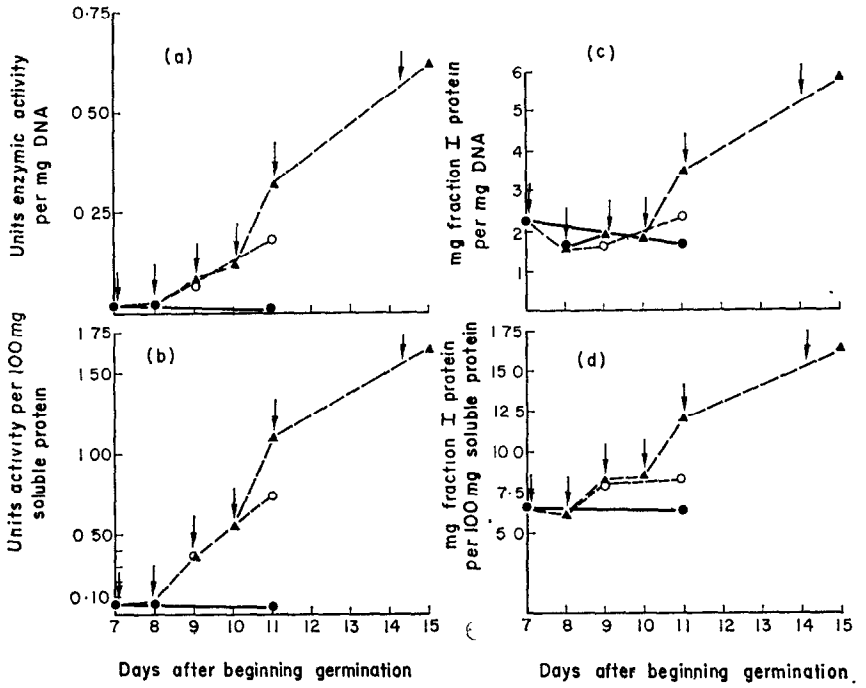

FIG. 3. EFFECTS OF SINGLE OR MULTIPLE IRRADIATIONS WITH RED LIGHT ON THE ACTIVITY OF RuP CARBOXYLASE (a AND b) AND AMOUNT OF FRACTION I PROTEIN (c AND d) PER UNIT DNA AND PER UNIT SOLUBLE PROTEIN.

The experiment is the same as Expt. 1, described in Fig. 1. All samples, except the last, were taken immediately before each red irradiation which is indicated by an arrow. Red irradiated as indicated by arrows, ▲; single red irradiation as indicated by first arrow, ○; dark controls, ●.

TABLE 1. EFFECTS OF A SINGLE RED LIGHT TREATMENT ON GROWTH IN ETIOLATED PEA STEM APICES

	Days after beginning of germination				
	Dark controls		Red irradiated		
Growth measurement	7	11	8	9	11
mg DNA/100 apices	—	0·72	—	—	1·7
g fresh wt./mg DNA	0·51	0·37	0·42	0·28	0·40
mg soluble protein/mg DNA	34·5	26·8	26·0	20·5	27·0

Data refer to the experiment shown in Fig. 3 in which a single irradiation with red light was given on day 7.

The effect of a single red irradiation on RuP carboxylase activity is shown in Figs. 3a and 3b. There is no difference between the single and multiple irradiation treatments until the fourth day after the initial red irradiation. Similarly, the changes in amounts of Fraction I protein follow closely those occurring in plants given multiple irradiations, except that the rise evident between the 10th and 11th day does not occur. Therefore, a single red irradiation when measured on a DNA or soluble protein basis is as effective as a daily irradiation up to the fourth day.

The Involvement of Phytochrome in Enzyme Synthesis

It was previously shown[5] that in etiolated pea stem apices the induction of enzyme synthesis by R was under the control of the phytochrome system. More comprehensive results on some growth parameters and three respiratory and three photosynthetic enzymes are shown in Table 2. The effects of red, red followed immediately by far-red or FR alone on these components are compared on a unit DNA and on a per apex basis. Results on a unit souble protein basis are essentially similar in overall pattern to those on a unit DNA basis.

TABLE 2. EFFECTS OF R, R FOLLOWED BY FR, OR FR ON GROWTH AND ON THE ACTIVITIES OF SOME RESPIRATORY AND PHOTOSYNTHETIC ENZYMES IN ETIOLATED PEA STEM APICES

	per mg DNA				per 100 apices			
Light treatment:	Dark	R	R + FR	FR	Dark	R	R + FR	FR
Growth parameters:								
Fresh wt (g)	0 716	0 481	0 551	0 506	0 311	1 580	0 775	0 495
Soluble Protein (mg)	44 1	31 0	36 5	33 3	19 0	102	51 4	32·6
mg DNA	—	—	—	—	0 433	3 290	1 398	0 986
Respiratory enzymes:								
NADP-isocitrate dehydrogenase	4 24	1 64	2 43	2 93	1 83	6 45	3 4	2 9
NADP-malate dehydrogenase	214	86	126	118	93	338	177	117
Enolase	8 3	3 1	4 9	4 95	3 6	12 0	6 9	4 9
Photosynthetic enzymes:								
NADP-G-3-P dehydrogenase	0 46	1 79	2 65	1 15	0 20	7 00	3 70	1 14
Alkaline fructose-1,6-bisphosphatase	0 32	0·54	0 31	0 27	0·14	2 11	0 44	0 27
RuP carboxylase	0 030	0 34	0 31	0 21	0·012	1·09	0 43	0 20
Fraction I protein (mg)	2 0	2·45	3·5	2 8	0·96	11 2	4 9	2 77

Irradiations with R (5 min), R (5 min) followed immediately with FR (20 min) or FR (20 min) alone were given daily for 5 days beginning with 7-day old dark-grown pea seedlings and the plants returned to darkness. Stem apices were harvested on the 12th day after the beginning of germination (i.e. after 5 daily irradiations) Controls were maintained in darkness throughout.

On a per apex basis, red irradiation resulted in increases in DNA, soluble protein and fresh weight of 7·6-, 5·4- and 5·1-fold, respectively, and increases of 3·3- to 3·6-fold in the activities of the respiratory enzymes NADP-isocitrate dehydrogenase, NAD-malate dehydrogenase and enolase. The photosynthetic enzymes alkaline fructose-1,6-bisphosphatase, NADP-G-3-P dehydrogenase and RuP carboxylase, showed much greater increases, namely 15-, 35- and 91-fold, respectively. Fraction I protein increased 11·7-fold.

All of these increases were substantially diminished if FR immediately followed the R. It is concluded therefore, that phytochrome mediates the changes described. Furthermore, the changes in photosynthetic enzymes are disproportionately greater than would be expected from the extent of R induced growth of the stem apices.

When considered on the basis of unit DNA (or soluble protein), the results are less well defined. In general, changes in activities of the respiratory enzymes and the growth of parameters per unit of DNA (or per unit of soluble protein) show a decrease in the R-irradiated seedlings whereas the activities of photosynthetic enzymes show an increase. These changes presumably reflect a rapid cell division under R treatment. The differences in response of the growth parameters and respiratory enzymes on the one hand and the photosynthetic enzymes on the other suggests that the latter are substantially more responsive to phytochrome-mediated control.

Initial Effects of Red or White Light on RuP Carboxylase Activity

The short term effects on RuP carboxylase activity of irradiation of etiolated pea stem apices with R or white light are shown in Fig. 4. In this experiment batches of seedlings were either exposed to white light continuously or to a single 3-min period of R and returned to darkness. A short period of either R or white light resulted in an approximately 2-fold increase in activity of the enzyme which increased slightly during the following 12 hr in continuous white light but declined slowly to the level of the dark controls in R seedlings. During the next 12 hr increases in enzymic activity were similar to those obtained in the experiment reported in Fig. 3. During the first 12 hr following the initial irradiation there was no detectable increase in Fraction I protein but in the subsequent 12 hr a small but significant increase was detected.

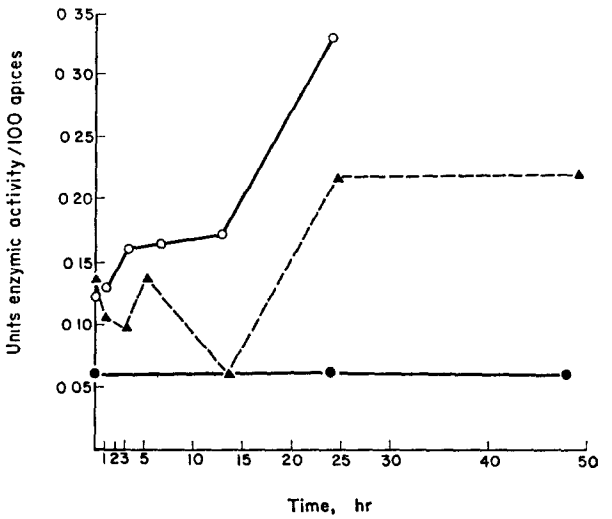

FIG. 4. EFFECT OF A BRIEF PERIOD OF RED LIGHT OR CONTINUOUS WHITE LIGHT ON THE ACTIVITY OF RuP CARBOXYLASE.

Seven-day-old etiolated seedlings were exposed to R for 3 min and returned to darkness. Other batches were illuminated continuously with white fluorescent light (8000 lx). Samples were taken at 3 min and at intervals thereafter. Harvesting and extraction was completed within 7 min after removal of the plants from the particular condition. Extracts were centrifuged at 1000 *g* for 5 min and assayed immediately for RuP carboxylase activity. Red irradiated, ▲; white illuminated ○; and dark controls, ●.

DISCUSSION

The results reported in this paper show that phytochrome mediates in the R-induced plastid development in etiolated pea stem apices. These results support and extend those reported previously.[5] Some photosynthetic enzymes of the chloroplast show increases of 91-fold (RuP carboxylase), 35-fold (NADP-G-3-P dehydrogenase) and 15-fold (alkaline-1,6-bisphosphatase) on a per apex basis after 5 brief, daily irradiations with R (Table 2). These increases are much greater than those of growth, whether measured as fresh weight or soluble protein (Table 2) or as DNA per 100 apices (Table 2 and Fig. 1), which show increases of 5·1- to 7·6-fold after four or five daily irradiations with low intensity R. Increases in the activities of some cytoplasmic enzymes are about 3·5-fold (Table 2) and are,

therefore, correlated with the growth changes. All these effects of R are largely prevented by FR immediately following R implicating phytochrome as the mediator of the effects (Table 2).

The time courses of growth measured as DNA per apex (Fig. 1) and fresh weight (Fig. 2a) or soluble protein (Fig. 2b) per unit of DNA show that there is a period of cell division for about three days following the initial R. By the fourth day of treatment with R (11th day after the beginning of germination) the cell expansion phase is underway. Soluble protein and fresh weight per cell increase during this phase of growth. The growth which occurs after R represents a substantial proportion of that attainable by the fully green tissue. (Figs. 1, 2a and b). It has been shown that R also induces synthesis of chloroplast ribosomal RNA and that this synthesis is under the control of phytochrome.[12]

The question arises whether the increases in plastid enzymic activities are a reflection of the phytochrome-mediated growth of the tissue. Compared with the growth parameters and activities of some respiratory (cytoplasmic) enzymes the increases in the photosynthetic enzymes were considerably greater when measured on a stem apex basis (Table 2). When examined on a unit DNA basis, which is assumed to be closely analogous to a unit cell basis, the growth parameters and respiratory enzymes do not show a classical phytochrome-mediated response probably because of the relatively high rates of cell division and growth, whereas the photosynthetic enzymes do (Table 2). A possible exception to this conclusion may be NADP-G-3-P dehydrogenase. This photosynthetic enzyme may show a light-activation response in addition to a phytochrome-mediated one but the kinetics[13] and action spectrum[14] make it unlikely that such light-activation could completely account for the present results. It is apparent, therefore, that some chloroplast enzymes behave in a manner different from those of the rest of the cell and from cellular growth. There is a real increase in activity of these photosynthetic enzymes per cell and such increases are apparently mediated by phytochrome.

It is conceivable that there could still be a constant level of, say, RuP carboxylase per etioplast in the dark-grown seedlings but that R may induce an increase in numbers of etioplasts per cell as well as the observed increase in DNA per apex. Except for studies of bean,[15] there is no information available regarding the number of etioplasts in leaf cells. In bean during 45 hr of greening of etiolated seedlings there was no increase in number of etioplasts per cell nor of cells per leaf. However, this observation may not apply universally since it has recently been shown[16] that an increase of about 5-fold in chloroplast number per cell occurs in spinach leaves during growth for 10 days under normal lighting conditions. During this time leaf area increased from 1 to 50 cm^2. After R induction in our experiments such an increase in number of plastids per cell accompanied by an approximately 7-fold increase in the number of cells (measured as DNA, Table 2) would give about a 35-fold increase in number of plastids per apex. This is of the same order of increases as found for various photosynthetic enzymes after 5 days of R when compared with a dark control (Table 2). Whether or not an increase in etioplasts number per cell accounts for the increases

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in photosynthetic enzymes, it is apparent that phytochrome is mediating a greater effect on chloroplast development than on the cytoplasmic enzyme systems and growth generally. Possible mechanisms whereby phytochrome may be involved in chloroplast development have also been outlined by Smillie and Scott.[17]

The effects of R on the activity of RuP carboxylase (Fig. 3) indicate that apart from the low level of R-induced rapid activation of RuP carboxylase discussed below, there is a lag of 24 hr before a substantial increase in enzymic activity is found. Therefore, this enzyme is quite different from the case of phenylalanine ammonia lyase which shows a detectable change in the rate of synthesis within an hour or two of irradiation.[2] In the case of the etiolated pea plant, new chloroplast ribosomes may have to be synthesized following R irradiation[12] before substantial chloroplast protein synthesis can occur.

Daily brief exposures to R do not have any greater effect on RuP carboxylase activity for 3 days than a single initial exposure (Fig. 3). An explanation of this observation could be that the initial exposure 'saturates' the phytochrome-mediated system involved in the increases so that further daily irradiations do not result in additional increases. However, a change occurs with the 4th daily treatment which results in a response greater than that found in plants given the single initial treatment. It is interesting that this time coincides with an increased rate of production of soluble protein (Fig. 2b).

There is an interesting contrast between the behaviour of Fraction I protein and its associated enzymic activity, RuP carboxylase, when considered on a soluble protein basis, following a single or multiple daily irradiations with R (Fig. 3). The level of Fraction I protein is relatively high in the dark controls in which the enzymic activity is exceedingly low. Following two irradiations with R the enzymic activity increases substantially while the amount of Fraction I protein increases but little, even after 3 irradiation treatments. At least 4 daily treatments with R were necessary to achieve a substantial increase in the level of Fraction I protein. Thus it appears that, on a soluble protein and also on a per stem apex basis (Table 2) a significant proportion of the Fraction I protein can be synthesized in the dark whereas the development of the enzymic activity of this protein requires R irradiation. Over longer periods of daily red irradiations synthesis of the protein is, however, closely paralleled by increases in enzymic activity.[5] Some possible reasons to account for the differences in behaviour of Fraction I protein and its associated enzymic activity have already been discussed elsewhere.[12]

Activation of RuP Carboxylase

The activation of RuP carboxylase by continuous white illumination shown in Fig. 4 is in agreement with that previously reported.[18] In our experiments no increase in Fraction I protein per stem apex was detected until 24 hr after the initial irradiation. The activation obtained with a single brief period of very low intensity R, also shown in Fig. 4, is of particular interest since it may be indicative of an immediate response by a phytochrome-mediated system. However, this response is exceedingly variable mainly due to variation in the low activity of the dark controls and attempts to test the FR reversibility of the response have not so far been successful. Recent observations of Criddle and his coworkers[19,20] indicate the presence of a light-activating factor for RuP carboxylase having

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maximal activity when the reaction mixture is illuminated with light at 325 nm. There are also significant stimulatory effects in the R and FR regions of the spectrum although the absorption and fluorescence spectra of the light-activating factor have spectral maxima at or below 450 nm. It is not clear, therefore, whether all the light stimulatory effects on RuP carboxylase activity can be attributed to light activating factor. The possibility remains that the photo-acceptor for the stimulation induced by R may be protochlorophyll(ide). The initial activation of RuP carboxylase, however, was very small in our experiments in relation to the subsequent increases in enzymic activity which are accompanied by significant synthesis of new Fraction I protein.

EXPERIMENTAL

Peas (*Pisum sativum*, var. Greenfeast; Yates Seeds, Sydney) were grown in vermiculite for 7 days in the dark at 25°. After the various irradiation treatments, samples of 1 to 2·5 g fresh wt. were obtained under a dim green safelight[21] by excising the stem apices immediately below the lowest leaf. Cell-free extracts were prepared as previously described.[5]

Irradiation treatments. Red (662 nm, half band-width 16 nm, 72 $\mu w/cm^2$) and far-red (733 nm, half band-width 10 nm, 20 $\mu w/cm^2$) light were obtained using a tungsten filament lamp with a glass cut-off filter (Corning c.s. 2–62) and interference filters (Schott and Gen, Mainz, Germany) White light was obtained from 'white' fluorescent lamps (8000 lx) either as continuous illumination or intermittently (16 hr day/8 hr night).

Analysis of extracts. Extracts were prepared for analysis of enzymic activity as previously described.[5] DNA content of extracts of pea stem apices was determined by a modification of the method of Smillie and Krotkov.[22] Soluble protein was determined in the cell-free supernatants by the method of Lowry *et al.*[23] using bovine serum albumin as standard.

Enzyme activities. RuP carboxylase (E.C. 4.1.1.39) was assayed by a method based on that of Smillie.[24] The reaction mixture, 0 6 ml, contained (μmoles per ml); tris, (pH 8·0); 125; $MgCl_2$, 50; EDTA, 1·3; 2-mercaptoethanol, 6·2; RuP, 1·67; $Na_2\ ^{14}CO_3$ + $KHCO_3$, 41·6 (1 $\mu c/\mu$mole C). Cell extract equivalent to about 7 mg soluble protein was activated with sulphydryl reagent at 25° for 10 min in the reaction mixture minus RuP and ^{14}C. ^{14}C was added and an activation period of 3 min allowed before starting the reaction with RuP. Samples (0·1 ml) were taken for up to 20 min and plated on Al planchettes containing about 0·3 ml of HCOOH–EtOH (1.1, v/v) and dried. Controls contained no RuP. Planchettes were counted with a Geiger end-window counter linked to an Ekco scaler. The reaction rate was linear for at least 10 min and showed no lag provided there was an initial activation period with $^{14}CO_2$.

NADP-G-3-P dehydrogenase (E.C. 1.2.1.13) was assayed by the method of Gibbs[25] but using higher concentrations of glyceraldehyde-3-phosphate (2 μmole/ml) and NADP (0·1 μmole/ml). NADP-isocitrate dehydrogenase (E C. 1.1.1.41) was assayed as described by Ochoa.[26] Malate dehydrogenase (E.C. 1.1.1.37) was also assayed as described by Ochoa.[27] Enolase (E.C. 4.2.1.11) was assayed by a modification of the method of Bucher[28] so that the reaction was started by the addition of 2-phosphoglycerate.

Estimation of fraction I protein. Polyacrylamide disc gel electrophoresis using the method of Ornstein and Davis[29] with 7·5% acrylamide was carried out on aliquot of the same supernatants used for enzymic assays. Extracts were run in duplicate with a loading of 200 μg protein per gel column. In samples in which Fraction I protein content was low, e.g. dark-grown and far-red irradiated peas, a loading of 400 μg protein per gel column was used. The gels were stained with Amido Black 10B in 7% (w/v) acetic acid and the amount of Fraction I protein was estimated by the method of Racusen and Foote.[30]

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