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THE EFFECTS OF LIGHT ON PLASTID RIBOSOMAL-RNA AND ENZYMES AT DIFFERENT STAGES OF BARLEY ETIOPLAST DEVELOPMENT

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Abstract—Light treatment accelerates the rate of ribosomal-RNA accumulation in apical sections of young etiolated barley leaves but has very little effect in older leaves. Light treatment, however, leads to increases of similar magnitude in the levels of $NADP^+$- and NAD^+-dependent glyceraldehyde phosphate oxidoreductases, alkaline fructose diphosphatase and Fraction I protein at all stages of leaf development. There are no effects of light on the specific activities of glucose-6-phosphate and isocitrate: NADP oxidoreductases in young leaves, but in older leaves marked decreases are observed. The results are discussed in relation to the mechanism of the photo-regulation of plastid enzyme levels.

INTRODUCTION

THE CONVERSION of the etioplasts of the dark-grown leaf into photosynthetically active chloroplasts requires a substantial period of irradiation with white light.[1] During this period of light treatment the levels of many photosynthetic enzymes have been demonstrated to increase markedly,[2-10] probably by *de novo* synthesis within the plastid.[2,6,10,11] The mechanism of this regulation by light is largely unknown, although evidence for the involvement of phytochrome has been reported.[9,10] Ingle[12] has reported that light increases the rate of plastid ribosomal-RNA synthesis in radish cotyledons by a factor of three. It could thus be postulated that the increased rate of plastid ribosome production may be a necessary step in the light-mediated increase in plastid enzymes. The experiments reported here were designed to test this possibility.

Smith[13] has shown that the levels of plastid ribosomal-RNA in apical sections of dark-grown barley leaves increase from near zero in the embryonic leaves to *ca.* 50 per cent of total ribosomal-RNA in 5–8-day-old leaves. Preliminary experiments indicated that white light increased plastid ribosomal-RNA levels markedly in young leaves, but had progressively less

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effect as the etiolated leaves aged. The effects of light on the levels of some plastid and cytoplasmic enzymes in apical sections of dark-grown barley leaves have therefore been investigated. Results suggest that the production of new plastid ribosomal-RNA is not an essential step in the light-stimulation of plastid levels, except in young leaves.

RESULTS

Effect of Light on the Levels of Plastid Ribosomal-RNA

Barley seedlings were grown either in the dark or given 24 hr white light 2, 3 and 4 days after soaking. At the end of the light treatments, 100 apical leaf sections (1 cm) from each sample were excised and the total nucleic acids extracted. Similar extractions were prepared from dark-grown controls. Equal quantities of nucleic acid (25 μg) were separated by polyacrylamide gel electrophoresis (Fig. 1). The relative proportions of plastid and cytoplasmic

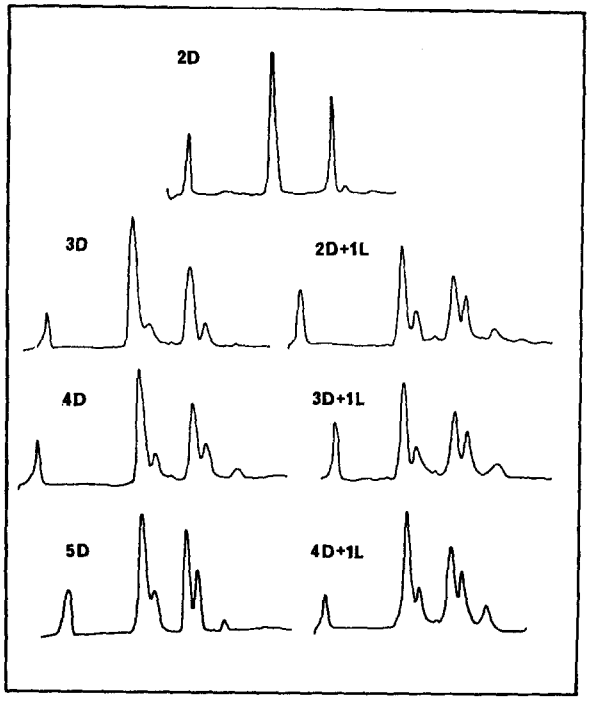

FIG. 1. POLYACRYLAMIDE GEL ELECTROPHORESIS OF TOTAL NUCLEIC ACID EXTRACTED FROM 1 cm APICAL SECTIONS OF LEAVES GROWN IN DARKNESS AND UNDER LIGHT TREATMENT.

Seedlings were grown in darkness and batches given 24 hr white light at 2, 3 and 4 days. The total nucleic acid was extracted by the phenol method and 25 μg separated on 2·6% gels for 3 hr.

D = number of days in darkness; L = number of days in light. (With 2-day-old specimens, whole leaves were taken, average length 0·7 cm.)

ribosomal-RNA were calculated by area measurement[12] and are presented in Fig. 2 as a function of leaf age. It is clear that the light treatment brings about a marked rise in plastid ribosomal-RNA levels in the young leaves, but that the effect is progressively diminished as the leaf grows. The levels of total nucleic acid per section decreased gradually as the leaves aged and were not significantly affected by the white-light treatment.

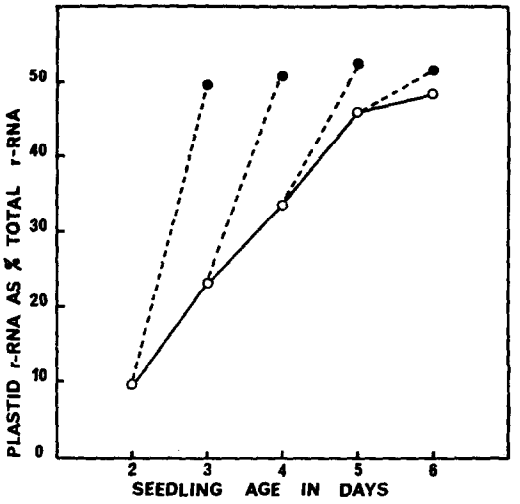

FIG. 2. EFFECTS OF 24 hr WHITE LIGHT AT VARIOUS TIMES AFTER SOAKING ON THE PROPORTIONS OF TOTAL RIBOSOMAL-RNA PRESENT AS PLASTID-RNA AT VARIOUS STAGES OF LEAF DEVELOPMENT.

The relative amounts of cytoplasmic and plastid ribosomal-RNA were calculated by area measurement of the original scans for Fig. 1. Open circles, dark-grown controls; closed circles, light treatments; dotted lines represent changes effected by light treatment.

Effects of Light on the Levels of Certain Plastid and Cytoplasmic Enzymes

The effects of the above treatments on some "plastid" and "cytoplasmic" enzymes are shown in Fig. 3, 4, 5 and 6. The plastid enzymes investigated were $NADP^+$- and NAD^+-dependent glyceraldehyde phosphate oxidoreductases and alkaline fructose-1,6-diphosphatase. The arguments for considering these enzymes as being restricted to, or concentrated in, the plastids have been discussed in the preceding paper.[13] The cytoplasmic enzymes

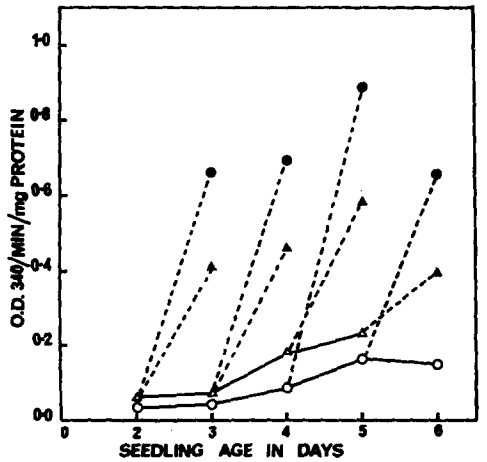

FIG. 3. THE EFFECTS OF 24 hr WHITE LIGHT ON THE SPECIFIC ACTIVITIES OF $NADP^+$-DEPENDENT (CIRCLES) AND NAD^+-DEPENDENT (TRIANGLES) GLYCERALDEHYDE-PHOSPHATE OXIDOREDUCTASES AT VARIOUS STAGES OF LEAF DEVELOPMENT.

Open symbols, dark-grown controls; closed symbols, light treatments; dotted lines represent changes effected by light treatment.

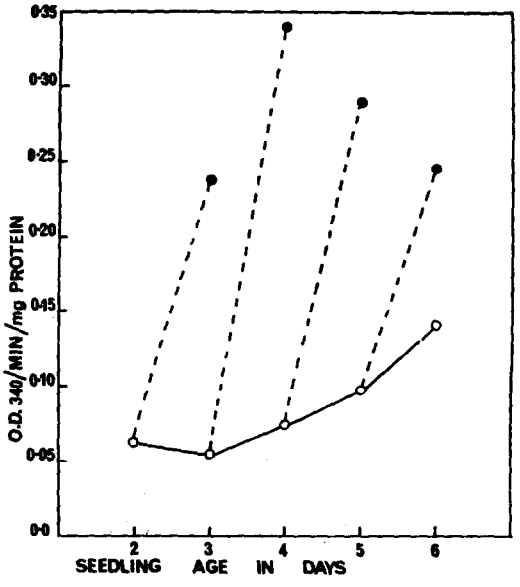

FIG. 4. THE EFFECTS OF 24 hr WHITE LIGHT ON THE SPECIFIC ACTIVITY OF ALKALINE (pH 8·5) FRUCTOSE DIPHOSPHATASE AT VARIOUS STAGES OF LEAF DEVELOPMENT.

Open symbols, dark-grown controls; closed symbols, light treatments; dotted lines represent changes effected by light treatment.

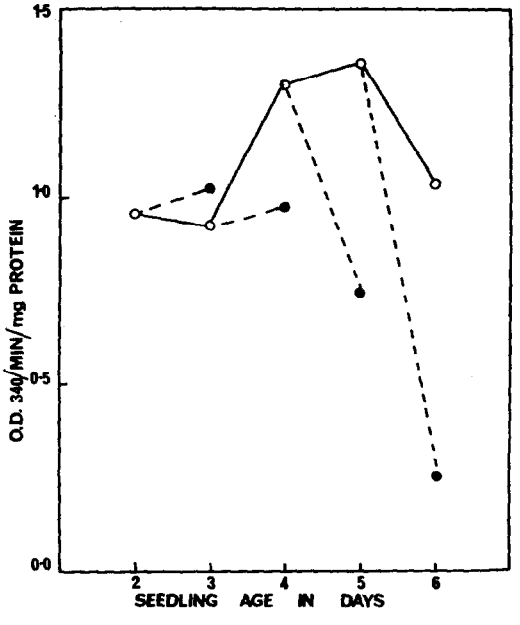

FIG. 5. THE EFFECTS OF 24 hr WHITE LIGHT ON THE SPECIFIC ACTIVITY OF GLUCOSE-6-PHOSPHATE: NADP OXIDOREDUCTASE AT VARIOUS STAGES OF LEAF GROWTH.

Open symbols, dark-grown controls; closed symbols, light treatments; dotted lines represent changes effected by light treatment.

investigated were glucose-6-phosphate: NADP oxidoreductase and isocitrate: NADP oxidoreductase.

The data in Fig. 3 illustrate that 24 hr of white light elicits increases in $NADP^+$-dependent and NAD^+-dependent glyceraldehyde phosphate oxidoreductase at all stages of leaf growth.

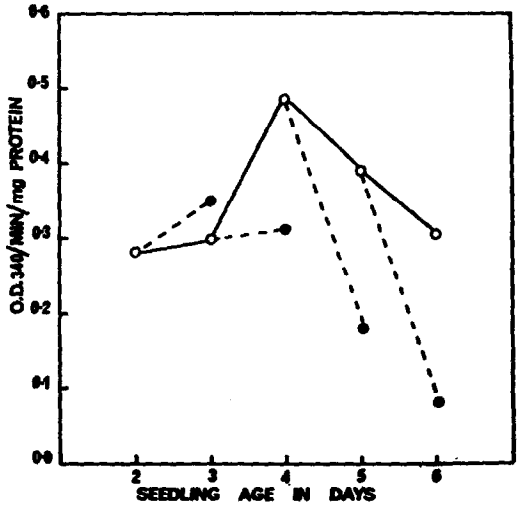

FIG. 6. THE EFFECTS OF 24 hr WHITE LIGHT ON THE SPECIFIC ACTIVITY OF ISOCITRATE: NADP OXIDOREDUCTASE.

Open symbols, dark-grown controls; closed symbols, light treatments; dotted lines represent changes effected by light treatments.

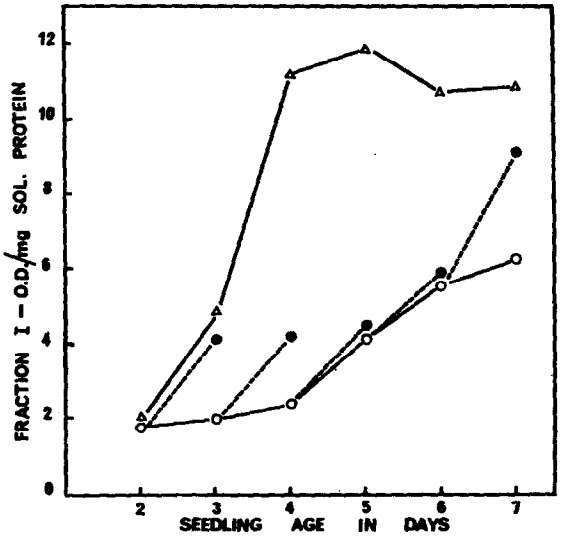

FIG. 7. THE LEVELS OF FRACTION I PROTEIN IN APICAL SECTIONS OF LEAVES GROWN IN DARKNESS, IN CONTINUOUS LIGHT, AND IN DARKNESS FOLLOWED BY 24 hr LIGHT GIVEN AT VARIOUS STAGES OF LEAF GROWTH.

Open circles, dark-grown controls; closed circles, leaves treated with 24 hr light at various stages; dotted lines represent changes effected by light treatment; open triangles, continuous light.

For both enzymes, the magnitude of the response to light does not differ greatly as the leaf ages. A similar pattern is seen in the case of alkaline fructose diphosphatase (Fig. 4).

With the cytoplasmic enzymes (Figs. 5 and 6) light treatment of young leaves had little effect whilst that of older leaves resulted in marked decreases in specific activity.

Effects of Light on the Level of Fraction I Protein

In order to assess the effect of light treatment on the levels of a major protein component of the plastids, the response of Fraction I protein levels to the above treatments was investigated. In this case also, the levels of Fraction I protein in apical sections of leaves grown under continuous illumination were assayed. It is clear from Fig. 7 that the effect of 24 hr light was similar at all stages of leaf development. Since considerable increase in Fraction I protein levels occurs in darkness from the fourth to the sixth day,[13] (Fig. 7), the effects of light during the period when this dark-increase was proceeding appear relatively small. In continuous illumination, however, considerably larger quantities of Fraction I protein were produced.

DISCUSSION

It is clear from these results that a 24 hr white-light treatment accelerates the rate of synthesis of plastid ribosomal-RNA in young barley leaves, and in this respect the responses are similar to those reported by Ingle[12] for radish cotyledons. However, since plastid ribosomal-RNA is synthesized at a steady rate during barley leaf growth in continuous darkness,[13] a stage is eventually reached where light treatment has no effect (Fig. 2). The proportion of total ribosomal-RNA present as plastid-RNA at this stage (*ca.* 50%) is similar to that in leaves grown for the same period in continuous illumination (H. Smith, unpublished results). Thus, as far as plastid ribosomal-RNA is concerned, light treatment merely accelerates the attainment of the state eventually reached in darkness.

A similar pattern is not observed with the plastid enzymes investigated. With $NADP^+$-dependent and NAD^+-dependent glyceraldehyde phosphate oxidoreductases and with alkaline fructose diphosphatase, light brings about increases in specific activity of similar magnitude at all stages of growth investigated. This is also true of Fraction I protein, and here it can be concluded that considerable net protein synthesis is occurring. In all these cases, however, if the increases brought about by light treatment are expressed as a percentage of the dark values at the start of the light treatment, the apparent light effect decreases with age. Thus, for $NADP^+$-dependent glyceraldehyde phosphate dehydrogenase, light at day 2 brings about a 30-fold increase in specific activity whilst, at day 5, the apparent effect is reduced to *ca.* 5-fold, although the absolute increases are roughly similar (Fig. 3). This is obviously due to the increase in specific activity during dark growth, but it illustrates the fallacy in placing emphasis on the proportional effects of light treatment. The inhibitory effects of light on the levels of the cytoplasmic enzymes are intriguing, but no attempts have been made to investigate them in detail. It is possible that these effects are also spurious in that the decreases in specific activity may be due to large increases in soluble protein levels, rather than to specific effects on the enzymes *per se*.

Various investigators have shown that the light-mediated increases in plastid enzyme levels are sensitive to inhibition by chloramphenicol.[2,6,10,11] This has been taken as evidence that enzyme synthesis is involved, and that this synthesis occurs on plastid ribosomes. These results are consistent with, but probably not exclusive to, this hypothesis. Thus, the light-mediated increases in the plastid enzyme levels occur both in etioplasts containing large

amounts of ribosomal-RNA (and therefore, presumably, large numbers of ribosomes) and in those containing very little ribosomal-RNA but, in the latter case, they are associated with marked increases in the plastid ribosomal-RNA levels.

These results, therefore, demonstrate that the synthesis of new plastid ribosomes is not an essential step in the regulation by light of plastid enzyme levels since the increases in the enzymes in the older leaves are not accompanied by increases in plastid ribosomal-RNA levels. It may be speculated however that, since the enzymes are produced at low, but steady, rates in the dark-grown leaves,[13] the messenger-RNA molecules for these enzymes are present in such leaves. It follows, then, that the regulation of enzyme levels by light may well be exerted at the ribosomal level, although quantitative effects on the rates of messenger production cannot be ruled out.

EXPERIMENTAL

Plant materials, culture conditions and light treatments. Seeds of barley (*Hordeum vulgare*, cv. Proctor) were obtained from Carters Seeds Ltd., London, soaked in running tap-water for 4 hr and sown in 5 cm of vermiculite. Seedlings were grown in a dark controlled temp. room at 25°. All manipulations were performed under a dim green safelight consisting of a 15 W daylight fluorescent tube filtered through three layers of Cinemoid No. 29 Primary Green (Strand Electrics, London). White-light treatments were given in a light-box containing sixteen 80 W, 1·5 m (5 ft) daylight fluorescent tubes *ca.* 1 m above plant height. Light intensity at plant height was 700 lux. In operations involving dark-grown leaves of less than 7–10 cm it was necessary to excise the leaves from the coleoptile.

Nucleic acid extractions and separations. Total nucleic acid was extracted by the tri-isopropyl-naphthalene sulphonate/4-aminosalicylate/phenol method of Kirby[14] as modified by Ingle and Loening.[15] Total nucleic acid was measured at 260 nm using an extinction coefficient obtained with yeast highly polymerized RNA (Sigma Chemical Co. Ltd.): $E^{1\%}_{1cm} = 220$.

Separation of total nucleic acid was performed by polyacrylamide gel electrophoresis on 2·6% gels.[16] In all cases 25 μg of purified nucleic acid was applied to each gel. Gel cylinders were scanned at 265 nm with a Chromascan recording densitometer (Joyce Loebl Ltd., Gateshead, England). The relative amounts of the different fractions of nucleic acid were calculated by the method described previously.[13]

Enzyme extraction and assays. Samples of 50–100 1-cm apical leaf sections were homogenized in 0·2 M borate buffer, pH 8·0, in a Virtis "23" at 23,000 rpm for 1 min. The homogenate was filtered through four layers of muslin and centrifuged at 30,000 *g* for 20 min. The supernatant was used as the enzyme preparation. All operations and storage of the preparations were carried out at 2–4°, and all assays were completed with 2 hr of preparation. In the cases of 2-day-old seedlings the average length of the leaves was 0·7 cm and consequently equivalent fresh wt of whole, excised leaves were used as experimental samples. The assays of glyceraldehyde-3-phosphate:NAD oxidoreductase, glyceraldehyde-3-phosphate:NADP oxidoreductase, alkaline fructose diphosphatase, glucose-6-phosphate:NADP oxidoreductase and isocitrate:NADP oxidoreductase were as reported previously.[13] Protein determinations were by the Lowry method.[17] The extraction, separation and quantitization of Fraction I protein was performed using the previously reported methods.[13]

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