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Phytochrome and Hormone Control of Polypeptides Synthesized by Chloroplasts of Senescent Barley Leaves

M. J. Quiles, J. Cuello and B. Sabater*

Departamento de Biología Vegetal
Universidad de Murcia
Murcia (Spain)

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To identify the polypeptides involved in the mechanism of leaf senescence, light-driven protein synthesis was assayed with chloroplasts isolated from barley leaf segments incubated during 20 h under different light and hormone treatments affecting senescence. The radioactive products were analyzed by SDS-PAGE and fluorography. The synthesis of some polypeptides was stimulated by ABA (66, 44, 30, 22, 20, kDa) and ethylene (66, 50, 48, 44, kDa) which accelerate senescence. Kinetin and red light (in an effect mediated by phytochrome), which retard senescence, inhibited the synthesis of some polypeptides (50, 48, 37, kDa) and stimulated the synthesis of others (54, 32, kDa). Probably phytochrome and hormones control senescence by affecting the synthesis of specific polypeptides.

Key words: Phytochrome, Polypeptides, Leaf senescence, Protein synthesis, Chloroplasts.

The retardation of leaf senescence by inhibitors of protein synthesis in chloroplasts (2, 11) suggests that senescence requires the synthesis of some specific proteins in chloroplasts, as well as in cytoplasm (8). Accordingly, chloroplast protein synthesis activity increases early and transitorily during natural (7) and detachment-enhanced (4) senescence.

Cytokinin treatment of senescent leaf segments (which inhibits senescence) changes the pattern of the polypeptides synthesized by isolated chloroplasts (4, 6).

Here, we analyze, by SDS-PAGE* and fluorography, the polypeptides synthesized by chloroplasts isolated from barley leaf segments treated during 20 h with different lights and hormones affecting leaf senescence (3).

* To whom all correspondence should be addressed: Departamento de Biología Vegetal. Universidad de Alcalá de Henares. 28871 Madrid (Spain).

* *Abbreviations.*— ABA, abscisic acid; PAR, photosynthetically active radiation; Pfr, far-red absorbing form of phytochrome; Pr, red absorbing form of phytochrome, SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

Materials and Methods

Barley (*Hordeum vulgare* L. cv Hassan) was grown on vermiculite, regularly watered with Cron medium (4) for 14 days under an 18 h photoperiod of white light (12.7 W m^{-2} PAR) at 24 and 16 °C day and night temperatures, respectively.

Three g of 20 mm sections, discarding base and tip, of the oldest leaf of 14-day-old barley, were incubated during 20 h at 24 °C with 20 ml pure water or 14 µM kinetin, 35 µM ABA or 70 µM ethylene (as ethrel). When indicated, dark incubation of leaf segments was interrupted for 10 min at 9 h with red light (3.2 W m^{-2}, 650 nm peak with 38 nm half-band width) or successive red, far-red (10 W m^{-2} of a wide band from 700 to 800 nm peaking near 800 nm) and red, far-red, red lights.

Chloroplasts were isolated as described by MARTÍN and SABATER (6). They were finally resuspended (to about 1 mg chlorophyll ml^{-1}) in 0.2 M KCl, 66 K-tricine, 6.6 mM $MgCl_2$, pH 8.3. Purified chloroplasts were, at least, 70 % intact and essentially free of mitochondria (< 5 % in protein) and cytoplasm (<1 % in protein) (6).

Light-driven protein synthesis by isolated chloroplasts was carried as described by MARTÍN *et al.* (7) in 450 µl incubation mixture containing 150 kBq of [^{14}C]-amino acid mixture (Amersham) (about 5 GBq $nmol^{-1}$) and around 250 µg chlorophyll, at 30 °C during 50 min. Radioactive proteins were precipitated with 5 ml of 8 % (w/v) trichloroacetic acid. The precipitate was resuspended for SDS-PAGE in 50 µl of 0.14 M Tris-HCl, 4.3 % (w/v) SDS, 11.1 % (v/v) mercaptoethanol, 33.3 % (v/v) glycerol, pH 6.8.

Slab SDS-PAGE was performed with the buffer system of O'FARREL (8) as described by MARTÍN and SABATER (6). Radioactive gels were impregnated with Amplify (Amersham) dried and fluorographed with Mafe RP-X-1 film exposed during 30 days at −80 °C. Parallel gel lanes with molecular weight markers were stained with Coomassie.

Chlorophyll was determined according to ARNON (1).

Results

Figure 1 shows that hormone treatment affecting senescence of detached primary leaves of 14-day-old barley affected (after 20 h treatment and before other effect on senescence was detected) the pattern of polypeptides synthesized by senescent chloroplasts. In agreement with previous results, some polypeptides (50, 48, 37, kDa) synthesized by chloroplasts isolated from leaves under accelerated senescence (lane 1) were not synthesized by chloroplasts isolated from kinetin-treated leaves (lane 4) which, specifically, synthesized

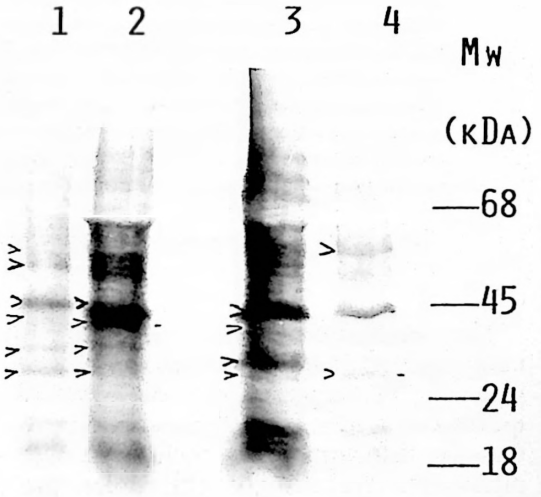

Fig. 1. *Polypeptides synthesized by chloroplasts isolated from leaf segments treated with different hormones.*
Leaf segments were incubated in the dark in pure water, 1; ethylene, 2; ABA, 3; or kinetin, 4. After protein synthesis, the polypeptides synthesized were analyzed by SDS-PAGE and fluorography. Marks (<) correspond to the main polypeptides cited in the text.

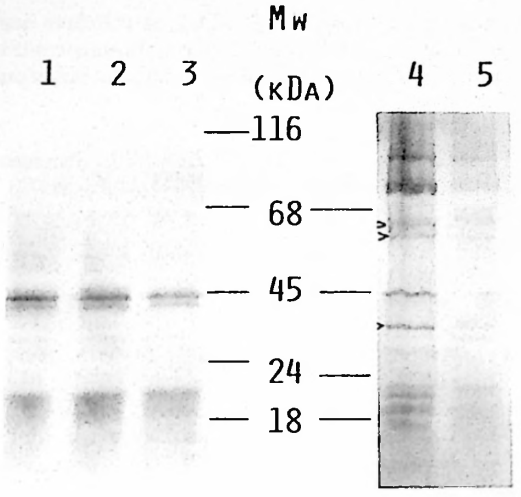

Fig. 2. *Polypeptides synthesized by chloroplasts isolated from leaf segments treated with kinetin and/or light pulses.*
Leaf segments were incubated in the dark in the presence of kinetin, 1 and 2; and/or dark interruption (10 min at 9 h incubation) with red lights, 2 and 3; red followed far-red followed red, 4; and red followed far-red, 5. After protein synthesis, the polypeptides synthesized were analyzed by SDS-PAGE and fluorography. Marks (<) correspond to the main polypeptides cited in the text.

(among other) the polypeptides of 54 (probably the large subunit of ribulose-1,5-diphosphate carboxylase) and 32 kDa. Enhancers of senescence showed a general effect stimulating the synthesis of senescence polypeptides. This stimulating effect was particularly notable for polypeptides of 66, 50, 48 and 44 kDa in chloroplasts from ethylene-treated leaves and polypeptides of 66, 44, 30, 22 and 20 kDa in chloroplasts of ABA-treated leaves.

Kinetin treatment or red light interruption of dark incubation retards senescence of leaf segment (2). The two effects are not additive (3) and the effect of red light interruption is mediated through phytochrome. Figure 2 shows that chloroplasts isolated from leaf segments incubated with kinetin and/or treated with a pulse of red light synthesized the same polypeptides (lanes 1, 2 and 3). Clearly, red light effect on chloroplast protein synthesis was mediated by phytochrome because subsequent far-red pulse (lane 5) changed the pattern of polypeptides synthesized by chloroplasts. Apparently, Pr form of phytochrome stimulated the synthesis (among other) of polypeptides typically synthesized by chloroplasts of enhanced senescent leaves (60, 58, 37, kDa, see also fig. 1). The synthesis of some of these polypeptides was inhibited by kinetin or Pfr form of phytochrome. Pr inhibited the synthesis of several polypeptides, in the range 52-56 kDa, induced by kinetin or Pfr. Subsequent red, far-red and red light treatments (lane 4) again changed, although in a complex way, the pattern of the polypeptides synthesized by isolated chloroplasts.

Discussion

Senescence acceleration in 14-day-old barley leaves by detachment and incubation in the dark is preceded by stimulation of the synthesis of senescence polypeptides in chloroplasts (4, 6). Senescence retardation by kinetin treatment of leaves is preceded by changes in the pattern of polypeptides synthesized by chloroplasts (4, 6).

The results shown in figs. 1 and 2 confirm previous results with kinetin and demonstrate that the actions of many other effectors on senescence are preceded by changes in the pattern of polypeptides synthesized in chloroplasts. It is tempting to suggest that several polypeptides synthesized in chloroplasts of senescent leaves determine subsequent senescence processes, because their syntheses are impaired by senescence inhibitors, as kinetin or red light pulse (50, 48, 37, kDa) or stimulated by senescence accelerators as ethylene (66, 50, 48, 44, kDa) or ABA (66, 44, 30, 22, 20, kDa).

Red light pulse effect on protein synthesis is clearly mediated through phytochrome (fig. 2). It is different from that of continuous light treatment which inhibits protein synthesis in chloroplasts of senescent leaf segments (6).

The results presented here and previous ones (4-6, 10) open the way to study the polypeptides and genes involved in the control of leaf senescence.

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Resumen

Se identifican los polipéptidos implicados en el mecanismo de la senescencia foliar, mediante la síntesis de proteínas dependiente de luz, con cloroplastos aislados de segmentos de hojas de cebada incubados durante 20 h bajo diferentes condiciones luminosas y hormonales. Los productos radiactivos se analizan por SDS-PAGE y fluorografía. La síntesis de algunos polipéptidos se estimula por ABA (66, 44, 30, 22, 20, kDa) y etileno (66, 50, 48, 44, kDa), que aceleran la senescencia. La kinetina y la luz roja, en un efecto mediado por fitocromo, inhiben la síntesis de algunos polipéptidos (50, 48, 37, kDa) y estimulan la de otros (54, 32, kDa). Se concluye que, probablemente, el fitocromo y las hormonas controlan la senescencia afectando la síntesis de polipéptidos específicos.

Palabras clave: Fitocromo, Polipéptidos, Senescencia foliar, Síntesis proteica, Cloroplastos.

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