THE INFLUENCE OF LIGHT OF DIFFERENT SPECTRAL REGIONS ON THE SYNTHESIS OF PHENOLIC COMPOUNDS IN GHERKIN SEEDLINGS, IN RELATION TO PHOTOMORPHOGENESIS IV. MECHANISM OF FAR-RED ACTION

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SUMMARY

It is shown that for an irradiation program of far red – darkness – far red the length of the lag-phases for respectively synthesis of hydroxycinnamic acids and induction of phenylalanine deaminase subsequent to the onset of the first and the second irradiation are of the same order of magnitude. This finding is discussed in relation to different hypotheses for the mechanism of far-red action in photomorphogenesis.

1. INTRODUCTION

The action of far-red light on phenol synthesis in seedlings has been interpreted in different ways. Following a hypothesis advanced by HARTMANN (1966), MOHR (1966) and WAGNER & MOHR (1966) explain the red, far-red reversible effect as well as the effect of prolonged exposure to far-red irradiation (high-energy reaction, HER) on the basis of the photo-reversible pigment phytochrome. The principal aspects of this hypothesis are that under prolonged exposure to far red a low but constant level of the unstable, active form of phytochrome (P 730) is maintained, and that this intermediate causes activation of particular genes. It is assumed that an activated gene will remain active as long as P 730 is present. One of the genes thus controlled would be that for phenylalanine deaminase (PADAse = phenylalanine ammonia-lyase) (DURST & MOHR 1966a, b), a key enzyme in phenol synthesis. In recent publications it is claimed that in mustard seedlings, if pre-irradiated and then kept in darkness for some time, a second irradiation with far red causes an immediate resumption of *de novo* synthesis of PADAse (RISSLAND & MOHR 1967), accompanied by a rapid increase in the rate of anthocyanin synthesis (LANGE, BIENGER & MOHR 1967). This is interpreted as evidence that genes can be activated by P 730 very rapidly, once a barrier has been removed by the preceding irradiation.

Investigations of the light-induced synthesis of hydroxycinnamic acids in gherkin hypocotyls have led to the conclusion, however, that the level of P 730 is unlikely to be the decisive factor in the HER (ENGELSMA 1967a, b). Instead the following hypothesis has been advanced. A photoreaction caused by the light absorbed by photomorphogenetic pigments gives rise to metabolic changes

leading to, among other things, the production of an inducer for the enzyme PADAse. The production of the inducer is a function of the number of light quanta absorbed, and in its turn the rate of *de novo* PADAse synthesis is a function of the amount of inducer supplied. The synthesis of PADAse is subject to repression by the end-products of the reaction, viz. cinnamic acid and p-coumaric acid (ENGELSMA, in preparation). An increase in the PADAse level gives rise to an increase in the production of these compounds and, in due course, to enhanced repression. Under conditions of repression a rapid inactivation of PADAse occurs (ENGELSMA 1967c; see also DURST & MOHR 1966b; SCHERF & ZENK 1967b; ZUCKER 1967). Induction, repression, and inactivation are coupled in such a way that a new equilibrium builds up at which the enzyme level remains more or less constant. Once such a new equilibrium has been reached a new increase in enzyme level can be induced only by such light treatment as will produce an amount of inducer sufficient to overcome the repression imposed by preceding irradiation.

It had already been shown that with blue light the respective lag-phases that followed the onset of successive light treatments had about the same lengths (ENGELSMA 1967a). The following experiment demonstrates that the same applies to far-red irradiations.

2. MATERIAL AND METHODS

Three-day-old gherkin seedlings (*Cucumus sativus* L., "Venlose niet plekkers", strain Tercken VI) grown in darkness at 25 °C were submitted to the following irradiation programs: a) 50 min far red followed by darkness, and b) 50 min far red, 9.2 hours of darkness, and then far red again. The amount of hydroxycinnamic acids per hypocotyl was determined as described in ENGELSMA & MEYER (1965) from duplicate samples of 25 seedlings each, harvested at intervals of different lengths as shown in *fig. 1A*. The PADAse level was determined as described in ENGELSMA (1967a) from samples of 100 seedlings each harvested at intervals of 1 hour (*fig. 1B*).

3. RESULTS AND DISCUSSION

Comparison of the curves of *fig. 1A* and *1B* appears to confirm that the PADAse level is the rate-determining factor for phenol synthesis in the gherkin hypocotyl. The increase in PADAse induced by the first light treatment becomes measurable after the irradiation has already been stopped, the changes in the enzyme level extending far into the subsequent dark period until a new equilibrium is reached. If a second light treatment is given at that point, a new increase in enzyme level is induced after a lag of about equal length as the one following the onset of the first irradiation. It seems likely that in this second irradiation the whole chain of dark reactions linking light perception to enzyme induction is involved once more.

These results are not in accord with those of RISSLAND & MOHR (1967) with

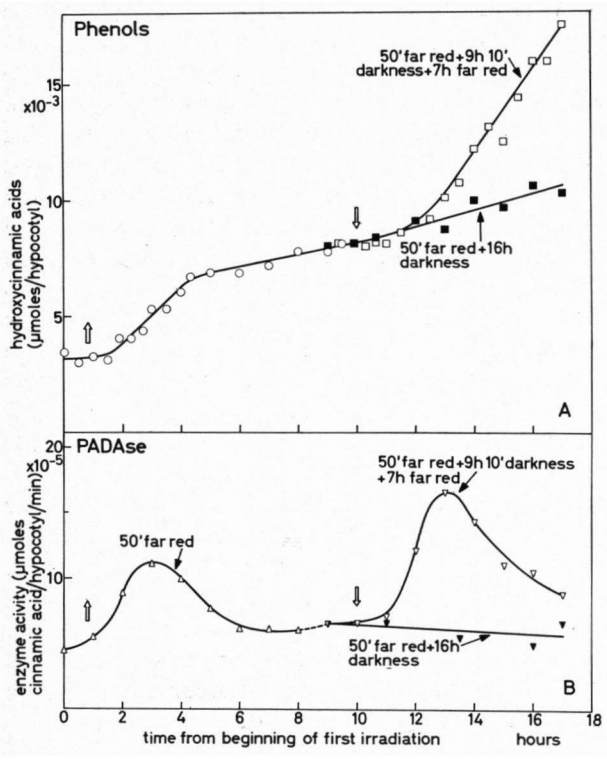

Fig. 1.
Accumulation of hydroxycinnamic acids (A) and levels of PADAse (B) in the hypocotyl of dark-grown gherkin seedlings under irradiation conditions as indicated. Intensity of the far-red light: 600 μW/cm². Arrows indicate the beginning and termination of the light treatments. Data collected in a particular experiment are represented by the same symbol, either open or closed.

mustard seedlings. The question then arises in how far the two experiments are comparable. From the curve shown in *fig. 1B* it may be inferred that if the intermediate dark period is made shorter, the changes induced in the PADAse level by first and second light treatments will interfere in a more complicated way. Thus a second lag-phase might become masked. Rissland and Mohr do not present a curve for the changes that occur after termination of their first light treatment and it therefore remains uncertain in which phase of those changes the second light treatment has been initiated.

As to the pigments involved in the HER, it seems to be well established now that the effects of blue light are not mediated by phytochrome (see e.g. HARTMAN 1967). A flavin or a flavoprotein seems to be the most likely candidate (PICKETT & FRENCH 1967; RAU 1967). The first step in the induction mechanism would then be a photoreaction sensitized by this pigment. Thus the observed energy dependence could be easily explained (ENGELSMA 1967a). It seems reasonable to assume that the high-energy effects of red and far-red light, which show a similar dependence on light intensity (ENGELSMA 1967b), are caused by a similar mechanism. In the latter case a photoreceptor that is structurally related to phytochrome or to chlorophyll and that sensitizes the same reaction as the blue-absorbing pigment is possibly responsible for the effect.

Evidence that, in addition to the pigments mediating the HER, phytochrome plays a role in phenol synthesis may be inferred from the fact that red, far-red reversible effects have been observed in a number of plants (DOWNS & SIEGELMAN 1963; GRILL & VINCE 1966; MOHR & VAN NES 1963; SCHERF & ZENK 1967a, b). In all cases mentioned in these references this latter effect manifests itself after a high-energy light treatment has first been given. Interpretations advanced for this phenomenon are that phytochrome depends for its action on substrate produced by the HER (GRILL & VINCE 1966) or that phytochrome interferes with the "expression into protein of genes that have been derepressed by the HER" (SCHERF & ZENK 1967a, b).

Recent experiments on light-dependent leaf movements indicate that changes in the state of phytochrome can cause the membrane permeability to change very rapidly (FONDEVILLE *c.s.* 1966; HILLMAN & KOUKKARI 1967; JAFFE & GALSTON 1967). This finding may offer an alternative explanation for the interaction between high-energy and red, far-red reversible effects. As stated above, the increase in enzyme synthesis induced by high-energy light is linked to the initiating photoreaction by a chain of dark processes. For a number of plants there is evidence that this involves, among other things, the transport of a promotive factor from one part to another (see ENGELSMA 1967a). Changes in membrane permeability that can be caused by phytochrome could have an effect on these processes and thus influence the enzyme induction. Such a mechanism would imply the possibility that under certain conditions rapid effects of light on the rate of enzyme synthesis could occur, without these effects having to be attributed to a specific gene activation.

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