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Involvement of Ethylene in Phytochrome-mediated Carotenoid Synthesis[1]

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ABSTRACT

Accumulation of carotenoid pigments in the shoot apex of etiolated pea (*Pisum sativum* cv. Alaska) seedlings is completely prevented by ethylene. Under certain conditions carotenoid synthesis is normally controlled by endogenously produced ethylene. The gas completely inhibits carotenoid synthesis induced either by continuous white light or brief illumination with red light, but only partially inhibits light-induced chlorophyll formation. Far red illumination followed by red illumination reverses the action of red light on carotenoid synthesis. Red light-induced carotenogenesis is partly or wholly caused by phytochrome-mediated inhibition of ethylene biosynthesis.

Phytochrome mediates photoinduced carotenoid synthesis in many tissues (10, 15, 24-27). Inhibition of ethylene production by red light seems not to be directly related to the primary action of phytochrome, but in certain cases, especially in etiolated dicot seedlings, ethylene functions as an intermediary between the primary action of phytochrome and the final manifestation of the responses to red light (6, 12, 16, 17). In the present communication, evidence is presented which indicates that ethylene is involved in phytochrome-stimulated carotenoid synthesis in etiolated pea seedlings.

MATERIALS AND METHODS

Plant Material. Seeds of *Pisum sativum* cv. Alaska were planted in wet vermiculite in plastic pots and placed in total darkness at 23 C. Approximately 100 seedlings were grown in each 12.5 cm diameter pot, and after various treatments the shoot apices (above the hook) were cut, weighed, and extracted.

Growth under Hypobaric Conditions and Gas Application. For hypobaric studies seedlings were placed in a sealed 9.5-liter desiccator, which was evacuated continuously at a controlled rate with a vented exhaust oil seal vacuum pump. Pure oxygen, water saturated at the lower pressure, was continuously admitted into the desiccator through a Matheson No. 49 regulator set to maintain the desiccator pressure constant at 120 mm Hg throughout the experimental period.

Seedlings grown at atmospheric pressure were treated with CO_2 or ethylene by injecting the gases into sealed desiccators containing pots of plants, to create appropriate gas concentrations. The desiccators were partially evacuated before introducing CO_2 to avoid creating a positive pressure, but they were returned to atmospheric pressure after the CO_2 was added.

Illumination. Except as indicated, experiments were carried out in total darkness using dim green light during handling of the plants. The red light source was a 40-w fluorescent lamp wrapped with several layers of DuPont red cellophane. Pots of plants were exposed to the red light (1250 erg cm^{-2} sec^{-1}) for 5 min and then placed in darkness thereafter. The far red light source was a 150-w tungsten bulb whose emission was passed through a Corning CS 269 filter to remove all visible light; the light intensity was 232 erg cm^{-2} sec^{-1} at the apex of the plants. For white light illumination, pots of plants were placed under a 22-w circline fluorescent tube for 18 hr.

Extraction and Measurements of Pigments. Tissue was ground with 90% methanol (10 ml/g fresh weight) with a Sorvall Omnimixer homogenizer at 5,000 rpm for 5 min. The resulting brei was passed through No. 52 Whatman filter paper, and the filtrate directly used for absorbance measurements with a Zeiss PMQ II spectrophotometer.

Total carotenoid content of etiolated tissue was determined by measuring the absorbance of the methanol extract at 442 nm. The alcohol extract had a typical carotenoid spectrum, with maximum absorption at approximately 442 nm and shoulder peaks at approximately 420 and 475 nm respectively. It displayed end absorption below about 400 nm, but this contributed little to the peak height at 442 nm. Moreover, observed changes in the absorbance of the carotenoid at 442 nm occurred without any concomitant change in end absorption.

The carotenoid content of green tissue exposed to white light was determined by a method modified from that of Kay and Phinney (18). An equal volume of methanolic potassium hydroxide (35 g KOH/100 ml methanol) was added to the methanol extract to saponify it in a separatory funnel. The same volume of petroleum ether was added to the mixture and it was gently shaken. Carotenoids were then partitioned into the petroleum ether layer by addition of saturated sodium chloride solution (twice the volume of the original methanol extract). The $A_{442\ nm}$ of the petroleum ether fraction was determined.

Total chlorophyll content of green tissue was determined by measuring the $A_{652\ nm}$ of the methanol extract (3).

RESULTS

Synthesis of carotenoids in the shoot apex is completely inhibited by ethylene. Concentrations as low as 20 nl/l produce a threshold response, 0.1 μl/l is half-maximal, and 1 μl/l is completely effective (Fig. 1). This concentration dependence curve is typical of other actions of ethylene (4, 6, 8).

The time course for ethylene inhibition of carotenoid accumulation in the dark is depicted in Figure 2. The data show carotenoids to be gradually accumulated in darkness unless

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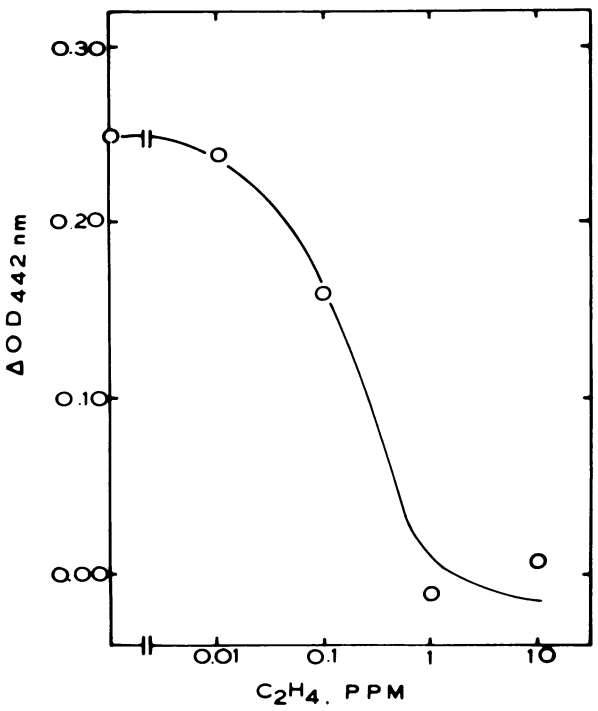

FIG. 1. Dose-response curve for the inhibition of carotenoid accumulation by ethylene during an 18-hr period in darkness. Treatments were applied to 7-day-old etiolated seedlings.

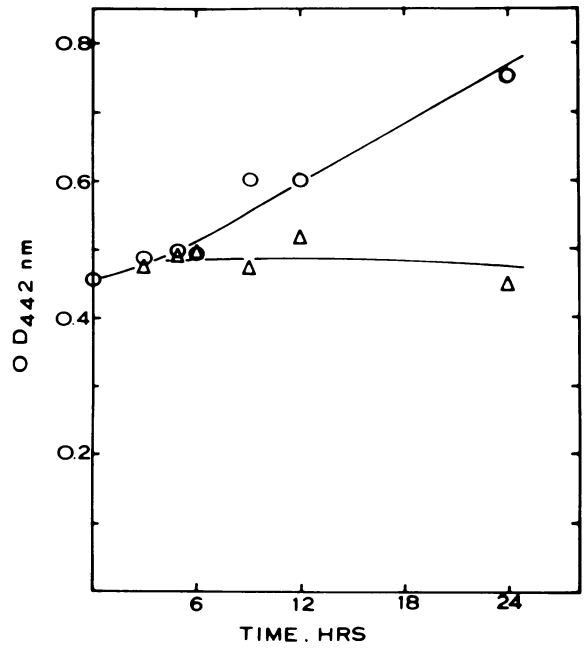

FIG. 2. The level of total carotenoids of the shoot apices in darkness with (△) and without (○) added ethylene (100 μl/l). Treatments were applied to 7-day-old etiolated seedlings.

ethylene is added, in which case accumulation is prevented. Almost no change in the carotenoid content of ethylene-treated plants occurs during a 24-hr period.

When plants are placed at a subatmospheric pressure, outward diffusion of endogenous ethylene from the tissue is facilitated, resulting in a lowered level of ethylene in the tissue even though the ethylene production rate is unchanged (7). Seedlings kept at 120 mm Hg of water-saturated oxygen grow normally, because under these conditions the partial pressure of oxygen is about equal to that in air at atmospheric pressure. However, seedlings grown in this manner for 4 days in darkness produce more carotenoids than controls grown under atmospheric conditions (Table I). In a shorter period (24 hr) the effect is smaller, but nevertheless shows the same tendency. Plants grown at a high concentration of CO_2, which antagonizes ethylene action (5), also produce slightly more carotenoids than control plants (Table I). These studies indicate that when endogenous ethylene is removed or displaced from its receptor, carotenoid synthesis is stimulated.

Red illumination reduces the rate of ethylene production in the etiolated shoot apex (6, 12), and the reduced content of endogenous ethylene is responsible for certain phytochrome-controlled photomorphogenic responses (6, 12, 16, 17). The data in Table II indicate that a 5-min exposure of seedlings to red

Table I. *Effects of Hypobaric Treatment and CO_2 on Carotenoid Synthesis*

Treatment	$\Delta A_{442\,nm}$	
	Experimental Period	
	24 hr[1]	96 hr
Control	0.235	0.390
120 mm Hg O_2	0.255	0.615
7% CO_2	0.265	

[1] The 24-hr period represents the interval between 7 and 8 days after planting; the 96-hr period represents the interval between 4 and 8 days.

Table II. *Enhancement of Carotenoid Synthesis by Red Light and Its Reversal by Far Red Illumination or Added Ethylene*

Treatment	$\Delta A_{442\,nm}$[1]
Dark	
Control	0.155
Ethylene, 100 μl/l	0.015
Red light	
Control	0.340
Ethylene, 100 μl/l	0.040
Far red (after red)	0.145

[1] Changes in $A_{442\,nm}$ after an 18-hr experimental period following treatment of 7-day-old etiolated seedlings.

Table III. *Effect of Ethylene on the Synthesis of Chlorophyll (652 nm) and Carotenoid (442 nm) in Seedlings Exposed to Continuous White Light*

Treatment	ΔA	
	442 nm[1]	652 nm
Control		
Dark	0.175	0.025
Light	0.665	0.352
Ethylene, 100 μl/l		
Light	0.000	0.200

[1] Changes in A after an 18-hr experimental period following treatment of 7-day-old etiolated seedlings.

light greatly increases the level of carotenoid present 18 hr later, and this effect is completely reversed by a subsequent 5-min illumination with far red light. In the presence of added ethylene, there is little, if any, new synthesis of carotenoid either in the dark or after red illumination; *i.e.*, the effect of red illumination is nullified by ethylene.

When etiolated seedlings are exposed to continuous white light, they soon start to synthesize chlorophyll in their plastids. The formation of this chlorophyll is only partially inhibited by ethylene (Table III). The data in the table indicate, however, that carotenoid synthesis under the same circumstances is completely prevented by ethylene.

DISCUSSION

There appears to be a lag period of about 6 hr before ethylene inhibits carotenoid synthesis (Fig. 2), which suggests that the effect of ethylene on carotenogenesis is rather indirect. Moreover, inhibition of carotenoid synthesis in response to ethylene is not a universal phenomenon. For example, in fruits ethylene promotes ripening and concomitantly, in many cases, an increase in carotenoid content (9, 13). Nevertheless, inhibition of carotenoid synthesis in pea seedlings appears to be a specific action of the gas. There is a close parallel between accumulation of carotenoid and chlorophyll in leaf tissue exposed to light (15, 25), and it has been suggested that light-induced carotenoid synthesis is merely one of the general phenomena which result from the formation of functional chloroplasts induced by light (14). However, the data in Table III show that under continuous white illumination, chlorophyll formation is only partially inhibited by ethylene, whereas carotenoid synthesis is completely inhibited. This indicates that ethylene inhibition of carotenoid synthesis is not due to an interference with plastid development.

The fact that carotenoid synthesis is enhanced by CO_2 and hypobaric conditions suggests that a changed endogenous ethylene content affects the rate of carotenoid synthesis. In etiolated pea shoot apices the rate of ethylene production decreases after brief red illumination (12), and the reduction in ethylene production causes expansion of the plumule (12) and opening of the apical hook (6, 16). As expansion of the plumule, opening of the apical hook, and carotenoid synthesis all are equally sensitive to ethylene (6, 12, 16), it follows that red illumination must stimulate carotenoid synthesis along with hook opening and plumular expansion when it inhibits ethylene synthesis.

Inhibition of ethylene production by red light may account for other photoresponses involving phytochrome, for there are several cases in which ethylene and red light cause opposite effects. For example, ethylene is a potent inhibitor of cell division in meristematic tissue (1), and the cell division frequency in etiolated seedlings of *Sinapis alba* is increased by the photomorphogenically effective radiation (22). If the apex of etiolated seedlings of *Sinapis alba* produces ethylene, and the production is inhibited by far red-absorbing phytochrome as is the case with other dicot seedlings (6, 12, 17), then a photoinduced reduction in ethylene production could in part or whole explain the increased frequency of cell division. Similarly, differentiation of metaxylem elements is inhibited by ethylene (2), and this process is to a certain extent induced by the phytochrome system (19). Ethylene prevents development of geotropic curvature by inhibiting lateral auxin transport (4), whereas geotropic reactivity is increased by red light in mustard seedlings (23). Brief illumination with red light induces anthocyanin synthesis in etiolated mustard seedlings (20, 21), whereas we find that in this tissue ethylene inhibits accumulation of anthocyanin when applied continually, just as it does if applied when anthocyanin accumulation is occurring (11). Thus in several cases a red light-induced reduction in the rate of ethylene biosynthesis may mediate the response to the phytochrome system.

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