ROLE OF CAROTENOIDS IN PROTECTING CHLOROPHYLL FROM PHOTODESTRUCTION[1,2]

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There have been numerous suggestions in the last few years of obligatory functions of carotenoids in photosynthetic organisms. These have been functions of carotenoids other than their action as accessory pigments. Workers who are interested in the mechanism of converting radiant energy into chemical energy frequently use the carotenoid molecule as a sink, or hole, in their photodissociation hypotheses (3, 12). Kohl (9) suggested many years ago that a function of carotenoids was to protect chlorophyll. Recently, evidence for such a role of carotenoids has been accumulating. Claes (2) has described four X-ray mutants of *Chlorella vulgaris* Bëyerinck. Three of these mutants do not form colored carotenoids and they are photosensitive. Sager and Zalokar (13) have studied a mutant of *Chlamydomonas rienhardi* Dang. which contains only trace amounts of carotene and which dies when exposed to light. It responds, however, to illumination by taking up carbon dioxide and releasing oxygen. Allen (1) also has shown that a carotenoidless mutant of *Chlorella pyrenoidosa* Chick is unable to grow in strong light.

The studies of Cohen-Bazire and Stanier (4) and Fuller and Anderson (6) have shown that colored carotenoids are required for protecting bacteriochlorophyll from photodestruction in the photosynthetic bacteria. It has been proposed that this is a unique role of carotenoids in all photoautotrophs (17). The results in the present communication support this hypothesis by extending to higher plants an experimental confirmation of the proposal through the study of an albino mutant of corn (white-3). Koski and Smith (10) have shown that this mutant forms protochlorophyll and chlorophyll; however, the chlorophyll is bleached upon continued illumination. An albino mutant of sunflower which reacts similarly has been described by Wallace and Schwarting (18).

MATERIALS AND METHODS

Plant materials used in these studies were grown under three different conditions. For carotene precursor studies, white-3 and normal corn seedlings were grown on a greenhouse bench for approximately ten days. Leaf material was ground in a mixture of acetone and hexane, and a hexane fraction containing carotene and colorless precursors of carotene was isolated by the method of Zscheile and Porter (20). Spectra of these fractions were measured with a Beckman DU spectrophotometer. Hexane was passed through a dry silica gel column to decrease absorbance in the ultraviolet by reagent grade hexane.

For the photostability of chlorophyll studies, the seedlings were grown in darkness for 8 to 12 days. Each determination consisted of six whole plants which were placed in 250 ml suction flasks. The flasks were evacuated in the dark two times; after each evacuation they were filled with either air or nitrogen. The plants in the flasks were illuminated with 1500 ft-c of light produced by a bank of tungsten filament lamps with an 8 inch water filter. After the exposure period, leaves of the plants were removed, weighed, and ground immediately in a mortar with 80 % acetone and a bit of sand. Ten ml of 80 % acetone was used for each gram of leaf tissue. Optical densities of the extracts were read at 665 mμ for chlorophyll and 630 mμ for protochlorophyll.

For some experiments the seedlings were grown in dim light with an intensity of less than 0.5 ft-c. Under these conditions, the white-3 seedlings were a blue-green color, and the normal seedlings a yellow-green. Both types of seedlings could be grown for two to three weeks under these conditions before they died.

RESULTS

ACCUMULATION OF PHYTOENE BY WHITE-3: The visible and ultraviolet spectra of the hexane extracts of normal and white-3 seedlings are presented in figure 1. The white-3 seedlings did not contain colored carotenoids as did normal seedlings. Instead, they had large amounts of substance which absorbed light in the ultraviolet region with peaks at 275, 285, and 297 mμ. These peaks are similar to those of phytoene (7), a precursor of colored carotenoids. Ergosterol, which has a spectrum similar to that of phytoene (7), was added during homogenization of white-3 corn seedlings, and after fractionation the hexane extract was poured through a neutral alumina column. The ultraviolet absorbing substance of the seedlings (phytoene) came through the column in the first 5 ml fraction, whereas added ergosterol appeared in the sixth 5 ml fraction when the column was leached with hexane.

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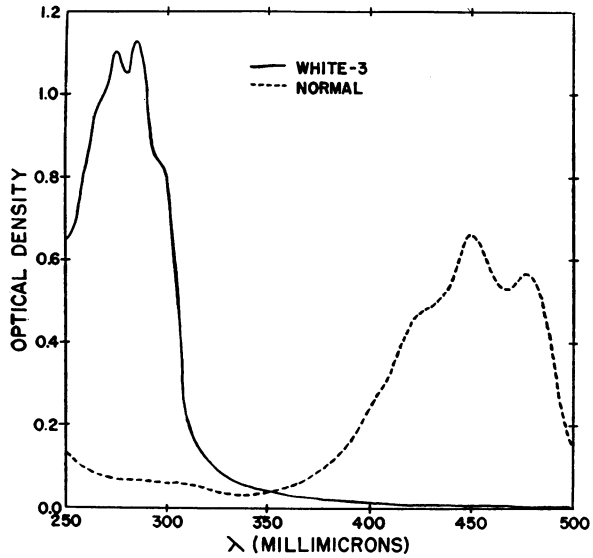
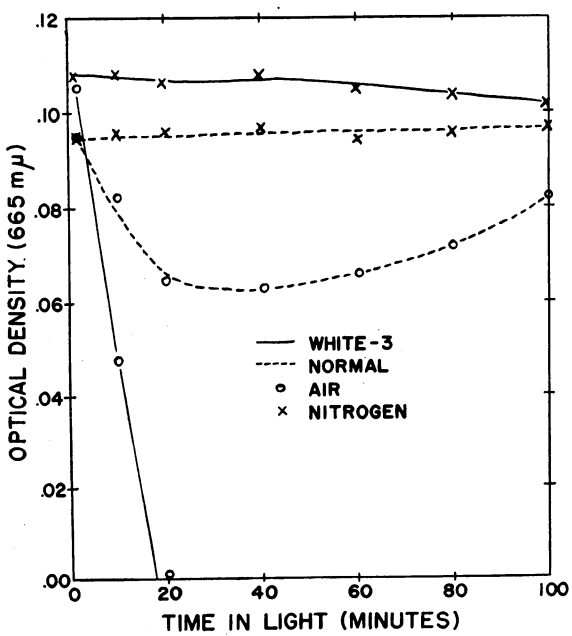


FIG. 1. Spectrum of hexane extracts from normal and white-3 seedlings.

PHOTOSTABILITY OF CHLOROPHYLL IN SEEDLINGS: As reported by Koski and Smith (10), dark grown mutant seedlings contained at least as much, usually more, protochlorophyll than did normal seedlings. The protochlorophyll of both types of genetic materials was readily converted to chlorophyll upon exposure of the seedlings to light. The results of exposing mutant and normal seedlings to light under various conditions are reported in figure 2. Chlorophyll of the mutant was completely destroyed when seedlings were illuminated in an atmosphere of air. Under anaerobic conditions, however, chlorophyll of the mutant was stable to high light intensities.

When leaves of normal seedlings were illuminated in air there was an initial decrease in chlorophyll content for the first 20 minutes, then a stabilization, and eventually an increase in chlorophyll content. It was suspected that this phenomenon might parallel the conversion of chlorophyllide a to chlorophyll a. Wolff and Price (19) have shown, in beans, that protochlorophyllide is photochemically reduced to chlorophyllide a, and that chlorophyllide a is esterified with phytol to form chlorophyll a in subsequent longer dark periods. Also with regard to this phenomenon, a study made by Granick (8) indicates a greater photolability of chlorophyllide a than of chlorophyll a. Some experiments were conducted with the corn seedlings, using the differential solubility of chlorophyllide and chlorophyll in the binary system of petroleum ether and acetone (19). These showed that most of the pigment which was formed by 1 minute of illumination was chlorophyllide; in a subsequent longer dark period the chlorophyllide was esterified to form chlorophyll. Nearly all the pigment from



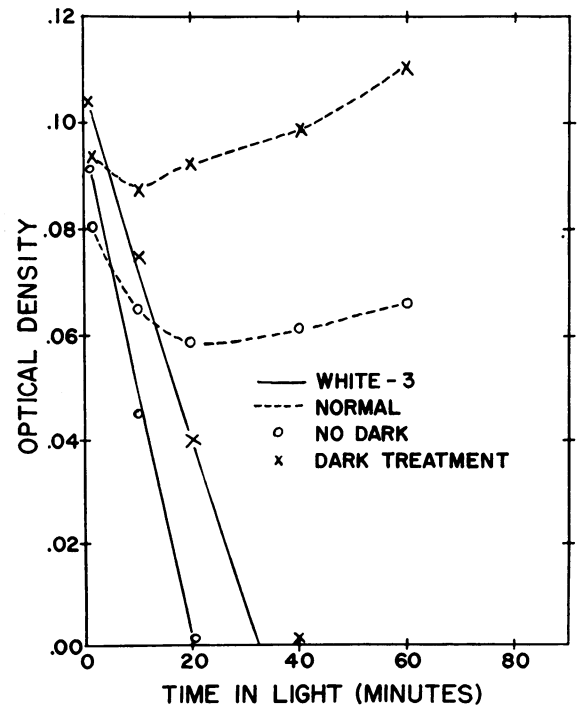


FIG. 2. Stability of chlorophyll of normal and white-3 seedlings as affected by exposure to light under aerobic and anaerobic conditions.

FIG. 3. Stability of chlorophyll a (dark treatment) and chlorophyllide a (no dark) of seedlings exposed to light in air.

both normal and mutant seedlings could be transferred to the petroleum ether phase after 30 to 40 minutes of darkness.

Since the previous experiments demonstrated that most of the pigment formed after 1 minute of illumination was chlorophyllide and that this was converted to chlorophyll during the dark treatment, any difference in the stability of pigment content to light in plants treated so as to contain mainly chlorophyllide or chlorophyll can be used to test the stability of these two forms of chlorophyll. Dark grown seedlings were illuminated for 1 minute and then either placed in the dark for 1 hour before being re-exposed to light or given no dark treatment and immediately exposed to further light. Pigment (chlorophyllide) in seedlings without a dark treatment was much less stable than the pigment (chlorophyll) in seedlings that had a dark treatment (fig 3). This was the most prominent during the first few minutes of illumination of normal seedlings. These results suggest that the non-esterified form of chlorophyll is less stable than the esterified form.

The continuous formation of protochlorophyll during the dark period after a brief illumination can be measured. It is illustrated also in figure 3 by the greater chlorophyll content of the plants receiving the dark treatment than those which did not receive the dark treatment. In fact, the small decrease in chlorophyll content during the first 10 minutes of illumination of the normal seedlings which had received the dark treatment probably represents a photochemical destruction of chlorophyllide a formed from the amount of protochlorophyllide which had accumulated during 1 hour of darkness.

It is of interest to consider the effect of anaerobic conditions on the formation of protochlorophyll and chlorophyll at the seedling stage of development. Anaerobiosis did not inhibit the conversion of preformed protochlorophyll to chlorophyll (fig 2). This has been reported previously by Smith (15). In this same figure, it is apparent that under anaerobic conditions there was no appreciable increase in the chlorophyll content of normal seedlings, whereas under aerobic conditions there was an appreciable increase in chlorophyll content between 40 and 100 minutes. The implication is that the formation of protochlorophyll requires oxidative metabolism. This is not unexpected if one considers that the citric acid cycle is the source of intermediates and energy for porphyrin synthesis (14).

Seedlings Grown With Dim Light: White-3 and normal seedlings were grown under a light intensity of less than 0.5 ft-c. This light intensity allowed for a continual increase in chlorophyll content over the 2 week period (table I). After 2 weeks of growth the seedlings were exposed to bright light for 1 hour in air. Nearly all the chlorophyll of the white-3 seedlings was destroyed by this treatment, whereas the chlorophyll of the normal seedlings was not appreciably effected by bright light.

A green pellet could be sedimented by 1000 × G from both normal and mutant materials when leaves were ground with sand in 0.35 M NaCl. Under the microscope, chloroplasts could be recognized readily in the pellet from the normal seedlings. The chloroplast-like particles in the pellet from mutant seedlings were rather opalescent but otherwise appeared to be normal. Leaves of the two types of seedlings were embedded in paraffin and sectioned. The chloroplasts of the mutant appeared to be as numerous and of the same size as those of the normal seedlings. Further studies are in progress on the number and size of chloroplasts and the capacity of chloroplasts from mutant seedlings to carry out the reactions of photosynthesis.

Table I

Accumulation of Chlorophyll by Seedlings Grown Under Dim Light and Its Stability to Bright Light

Seedling	Dim light		Bright light for 1 hr after 14 days dim light
	7 Days	14 Days	
	μg chlorophyll/g fresh weight		
Normal	19	61	58
White-3	12	28	5

Discussion

In this particular albino mutant of corn, chlorophyll and chloroplast formation appear to be normal. That is, protochlorophyllide of dark grown mutant seedlings is converted to chlorophyllide by short exposure to light. The mutant tissue has the capacity to esterify chlorophyllide to form chlorophyll. If grown under dim light conditions chloroplast development in the mutant appears to be similar to that of normal seedlings.

Chlorophyll of the mutant, however, is unstable to high light intensities in the presence of oxygen. Under strictly anaerobic conditions the chlorophyll of the mutant is stable to strong light. These results are similar to those reported for the carotenoidless condition in photosynthetic bacteria (4, 6). As in the bacteria, the instability of chlorophyll appears to be due to the lack of colored carotenoids in the mutant. These results, then, support Stanier's (17) prediction that colored carotenoids are required in all aerobic photoautotrophs for the protection of chlorophyll from autophotodestruction.

The function of carotenoid pigments for protection of chlorophyll from photodestruction may not be limited to photoautotrophs. It may have far wider significance than this. Eyster (5) has shown that the catalase activity of an albino mutant of corn is considerably lower than it is in normal seedlings. Mathews and Sistrom (11) recently showed that cells of a carotenoidless mutant of *Sarcina lutea* Schroeter are killed when placed in sunlight under aerobic conditions. Nearly all air borne pollen grains

and many air borne fungal spores contain carotenoids. It is entirely conceivable that carotenoids may protect other light absorbing porphyrins such as catalase and cytochrome from photodestruction.

We do not wish to imply that all albino mutants are caused by a metabolic block in carotenoid biosynthesis. There presumably is a different metabolic block in all the nonallelic albino mutants of a species. Consequently, there are many possible causes of albinism. For example, a metabolic abnormality that prevented normal chloroplast development could cause albinism. The studies of Smith (16) show, however, that many of the albino mutants of corn lack carotene. Our preliminary survey of the same group of mutants indicates that there are a number of metabolic blocks at different steps in the synthesis of carotenoids.

Carotenoids conceivably may have more than one obligatory role in higher plants, but a basic function is the protection of chlorophyll from photooxidation. In the obligately anaerobic photosynthetic bacteria there is no such strict requirement of carotenoids for the protection of chlorophyll from destruction, or for any other obligate role, since carotenoidless bacteria grow equally as well as do those containing carotenoids (4). In the aerobic photoautotrophs, on the other hand, the presence of colored carotenoids is vital for autotrophic growth in the light. Other obligatory roles of carotenoids may have evolved in higher plants along with the evolution of the more complex photosynthetic system of the chloroplast, in which oxygen is produced, compared to the non-oxygen producing chromatophore of the bacteria. The albino mutants seem to be very useful tools for further studies on the function of carotenoid pigments in photosynthesis.

Summary

There is a metabolic block between phytoene and colored carotenoid synthesis in white-3. Chlorophyll and chloroplast formation by the mutant appears to be normal, but its chlorophyll is destroyed by high light intensities under aerobic conditions. Under anaerobic conditions, however, the chlorophyll of the mutant is stable to strong light. The results support the thesis that an important role of colored carotenoids is the protection of chlorophyll from photodestruction.

Acknowledgment

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