A CHLOROPLAST ABSORBANCE CHANGE FROM VIOLAXANTHIN DE-EPOXIDATION. A POSSIBLE COMPONENT OF 515 nm CHANGES.[1]

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Summary. A light-induced chloroplast absorbance change that is stimulated by ascorbate and is due to violaxanthin de-epoxidation is described. The difference spectrum had characteristics of a carotenoid shift with a large positive peak at 505 nm, smaller peaks at 468 and 437 nm, and a sharp negative change at 483 nm. The absorbance change in the 510 to 520 nm region was appreciable, suggesting the possibility that de-epoxidation is a component of so-called 515 nm changes. The effects of DCMU[2], DPIP, and nigericin on the 505 nm change indicated that de-epoxidation was mediated by hydrogen-ion transport linked to photosystem 1.

Light-induced absorbance changes in the 515 nm region which appear to be due to shifts in carotenoid or chlorophyll absorbance and which apparently reflect a physical change associated with electron transport have been observed in leaves, isolated chloroplasts and green algae. (See reviews 1, 2.) Chlorophyll b has been shown to contribute to 515 nm changes (3) although not exclusively (4) and specific carotenoids which may contribute to these changes have not been determined.

Specific light-induced conversions of carotenoids are known. Violaxanthin (5, 6, 5', 6'-diepoxyzeaxanthin), a major carotenoid in green plants and algae, has been shown to undergo rapid and reversible light-induced conversion

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[2]Abbreviations used are: HEPES, N-2-hydroxyethylpiperazine-N'-2 ethane sulfonic acid; DPIP, 2,6 dichlorophenolindophenol; DCMU, 3-3(3,4-dichlorophenyl)-1, 1-dimethylurea.

to zeaxanthin through the monoepoxide antheraxanthin (5). This conversion in which oxygen is alternately lost (de-epoxidation) and re-incorporated (epoxidation) from O_2 (6) appears to be a cyclic pathway for photosynthetic O_2 uptake (7) mediated indirectly by photosynthetic electron transport (8). Recently Hager (9) concluded from the effects of pH and uncouplers that de-epoxidation was a reflection of chloroplast acidification from hydrogen-ion transport linked to photosystem 1 and therefore related to photophosphorylation.

Since, in organic solvent, zeaxanthin absorbs light at longer wavelengths than violaxanthin (10), it appeared possible that de-epoxidation in chloroplast could be detected as a carotenoid shift in a difference spectrum. This was found to be the case. In isolated chloroplasts, ascorbate and light stimulated a large absorbance change which had characteristics of a carotenoid shift. The factors which affected this absorbance change paralleled violaxanthin de-epoxidation activity determined by conventional pigment analysis.

Chloroplasts were prepared at 0° from market lettuce (Latuca sativa) commonly known as Manoa lettuce in Hawaii. About 30 g of chopped leaves were homogenized in 150 ml of SNH solution (0.4 M sorbitol, 10 mM NaCl, 50 mM HEPES, pH 7) for 5 sec in a semi-micro Waring blendor, filtered through 16 layers of gauze, and centrifuged at 500 x g for 5 min. The resulting pellet was resuspended in SNH solution and, for spectrophotometric studies, filtered through a layer of glass wool to remove large particles. Absorbance changes in chloroplasts were determined at 25° with a Perkin-Elmer Model 356 spectrophotometer. Light intensity was measured with a YSI Model 65 radiometer.

The light minus dark difference spectrum in the presence of ascorbate is shown in Fig. 1. The difference spectrum was characterized by peaks at 505, 468 and 437 nm with valleys at 483 and 447 nm. The change at 505 nm was a positive change whereas the position of the 468 nm was below the base line in some preparations. The peak at 437 nm was small and not always evident. After the light was turned off, the spectrum near 505 nm persisted but the

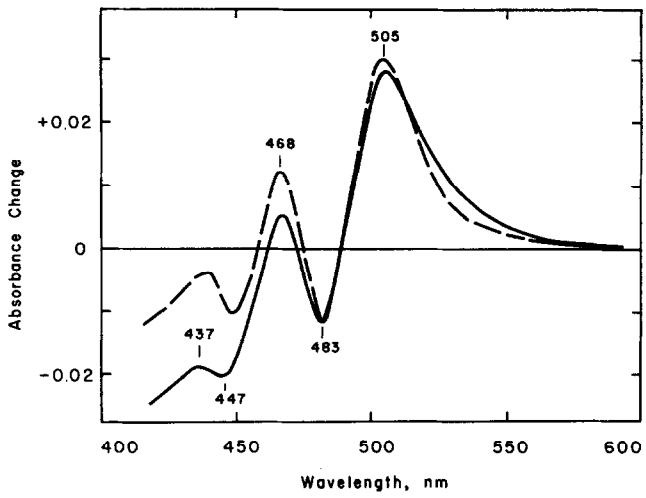

Figure 1. Light minus dark chloroplast difference spectra during (——) and after (---) continuous illumination. Chloroplasts (1.7 A at 680 nm) suspended in SNH solution containing 30 mM sodium ascorbate were illuminated with light filtered through red Corning filter CS2-58. The photomultiplier was shielded from actinic light with Corning filter CS4-96. The light intensity was 2×10^5 ergs cm^{-2} sec^{-1}.

Table 1

PROPERTIES OF VIOLAXANTHIN DE-EPOXIDATION IN ISOLATED LETTUCE CHLOROPLASTS DETERMINED BY CHROMATOGRAPHIC ANALYSIS.

Additions	De-epoxidation (mμ mole per mole chlorophyll)
Experiment 1	
Ascorbate	17
Ascorbate + DCMU	0
Experiment 2	
Ascorbate + DCMU	3
Ascorbate + DCMU + DPIP	11
Experiment 3	
Ascorbate	18
Ascorbate + DCMU	0
Ascorbate + Nigericin + KCl	1

Chloroplast suspensions (30 μg chlorophyll/ml) in 10 ml SNH solution were illuminated with broad-band red light (2×10^5 ergs cm^{-2} sec^{-1}) in air for 15 min, inactivated in hot methanol, and analyzed by thin-layer chromatography on Micro-Cel C. The concentrations of various additions were as indicated in Figure 2.

portion below 483 nm shifted upward, possibly due to other broad and reversible superimposed changes. Fig. 2 shows that the 505 nm change was stimulated by ascorbate and was inhibited by DCMU. Nigericin, a potent hydrogen-ion transport inhibitor (11), also inhibited the 505 nm change. DPIP partly reversed the DCMU inhibition. These results closely paralleled similar experiments on violaxanthin de-epoxidation followed by chromatographic analysis (Table 1). The ascorbate requirement and nigericin inhibition indicate that violaxanthin de-epoxidation is a reflection of both acidification from hydrogen-ion transport and reducing conditions. Reversal of DCMU inhibition by DPIP indicates that the hydrogen-ion transport which mediates violaxanthin de-epoxidation is linked to photosystem 1.

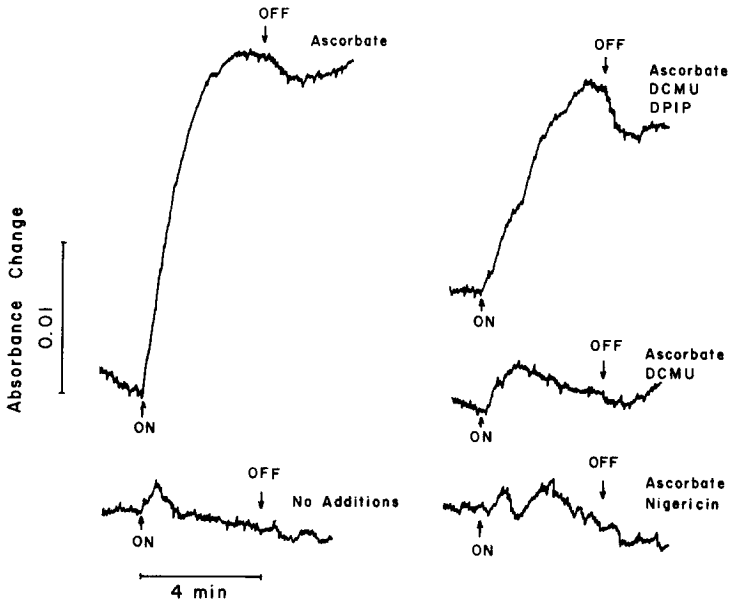

Figure 2. Kinetics of absorbance changes at 505 nm. The reference and sample wavelengths were at 580 and 505 nm respectively. When present, the concentrations were 30 mM sodium ascorbate, 10 μM DCMU, 50 μM DPIP, and 182 nM nigericin with 50 mM KCl. The absorbance of the suspension at 680 nm was 0.96. Other conditions were as described in Figure 1.

Further evidence that the chloroplast difference spectrum was derived from violaxanthin de-epoxidation is seen in the difference spectrum (zeaxanthin minus violaxanthin) of the isolated pigments in acetone shown in Fig. 3.

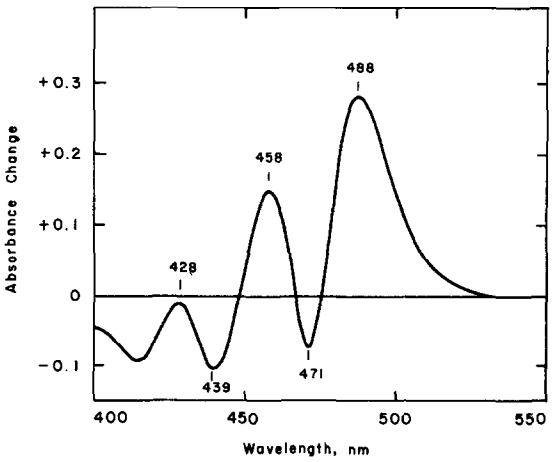

Figure 3. Difference spectrum of zeaxanthin minus violaxanthin in acetone. The pigments were isolated from lettuce by procedures described previously (5). The absorbance of zeaxanthin and violaxanthin was 0.56 at 452 nm and 0.58 at 443 nm respectively.

The shape of this difference spectrum is clearly similar to the light-induced change in chloroplasts, although the peak positions differ. The difference in peak positions is probably due to differences in the absorbance spectra of these pigments in acetone and in chloroplasts.

It is concluded that the observed light-induced absorbance change in the presence of ascorbate is derived from violaxanthin de-epoxidation. The extent of this change is large; at 505 nm the change is about 3.5% of the initial absorbance. Although the maximum absorbance change is located at shorter wavelengths than so-called 515 nm changes (12) the de-epoxidation absorbance change extends beyond the 515 nm region; hence it appears possible that absorbance shifts due to de-epoxidation could be a factor in 515 nm changes. Fork et al. (4) have shown that the 515 nm change is derived from chlorophyll b and at least one other component. Although in the presence of ascorbate the 505 nm change was not reversible, ^{18}O experiments have demonstrated reversible conversions of violaxanthin in leaves (7). Under reversible conditions de-epoxidation could give rise to transient absorbance changes similar to, but smaller than the ascorbate-induced change in chloroplasts.

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