ENERGY TRANSFER IN DEVELOPING CHLOROPLASTS

Warren L. Butler

Instrumentation Research Laboratory
Agricultural Marketing Service
United States Department of Agriculture
Beltsville, Maryland

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The resonance transfer of energy from photosynthetic accessory pigments such as carotenoids and phycobilins to chlorophyll a has been demonstrated (French and Young, 1951, Duysens, 1952) by showing that the absorption of light by such pigments results in fluorescence emission from chlorophyll a. The efficiency of resonance transfer depends on the degree of overlap between the emission spectra of the donor molecule and the absorption spectrum of the acceptor molecule and the proximity of the two molecules. The close proximity of accessory pigments to chlorophyll a is assured in the organized lamellar grana of mature chloroplasts from green plants. This organization of structure is not observed in the plastids of dark-grown plants, however. Electron micrographs of etiolated plastids (Hodge, McLean and Mercer, 1956, Muhlethaler and Frey-Wyssling, 1959) show the lamellar structures do not develop until after the plants have been exposed to light. The structural development appears to require chlorophyll synthesis (Wolken, 1959). In the data reported here, resonance transfer between carotenoid pigments and chlorophyll a does not occur immediately after the initial photoconversion of protochlorophyll to chlorophyll a but develops during a period thereafter. Resonance transfer develops as the carotenoid pigments and chlorophyll a are brought together in the organized structure of the developing lamellae.

Methods and Materials

A primary leaf harvested from a 6-day-old bean plant (Phaseolus vulgaris, var. Black Valentine) grown in complete darkness was spread

open to a single thickness and mounted between two ring-like discs with moulding clay. Resonance transfer between carotenoid pigments and chlorophyll a is indicated by the appearance of carotenoid bands in the excitation spectrum of chlorophyll a fluorescence. The fluorescence excitation spectrum is determined by placing cut-off filters (Corning filters 9830, 2030, and 2403) between the leaf and the phototube which transmit the chlorophyll fluorescence beyond 680 mμ but not the shorter wavelength exciting light. Monochromatic light from a scanning monochromator is incident on one side of the leaf while the phototube views the other side through the cut-off filters and records the fluorescence emission on a logarithmic photometric scale. These spectra have been corrected for the energy of the monochromatic exciting light expressed in Einstein's /cm^2/sec. Self-absorption of the fluorescent radiation is minimized by keeping the chlorophyll content low. Absorption spectra in the red region of the spectrum were obtained by removing the cut-off filters in order to measure the light transmitted by the leaf. The single-beam recording spectrophotometer used in this work has been described elsewhere (Butler and Norris, 1960).

Results

Fluorescence excitation and absorption spectra of a dark-grown bean leaf before and after brief exposures to light are shown in Figure 1. The spectra labeled A are due to protochlorophyll. The B spectra were obtained 1 minute after a 20-sec. light exposure which converted the protochlorophyll (abs. max. 650 mμ) to chlorophyll a (abs. max. 685 mμ). During the subsequent 10 minutes in the dark (curves C), the chlorophyll converted to a form which absorbs at 673 mμ as Shibata (1957) has shown. The excitation spectra indicate that the 673-mμ absorbing form is more highly fluorescent than the 685-mμ form. During a further dark period of 50 min. (curve D) bands ascribable to carotenoid pigments appear at about 470 and 500 mμ in the excitation spectrum. The 1-hour dark period is sufficient for maximal development of the carotenoid excitation bands in a 6-day-old leaf. The absorption spectrum D shows a resynthesis of protochlorophyll during this

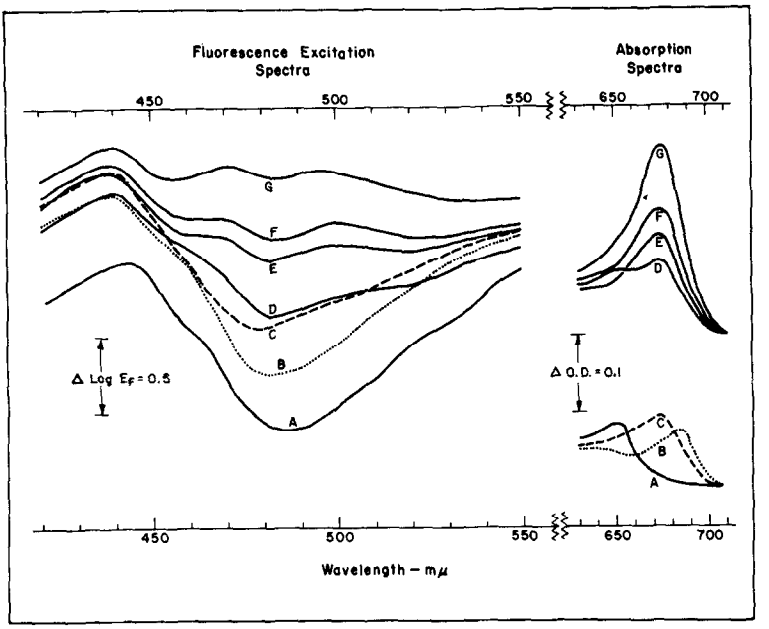

Fig. 1 Fluorescence excitation spectra and absorption spectra of a dark-grown bean leaf. A: before illumination, B: 1 min. after 20 sec. illumination, C: 10 min. after illumination, D: 1 hr. after illumination, E: 1 hr. after second 20 sec. illumination, F: 1 hr. after third 20 sec. illumination, G: 1 hr. after 2 hrs. continuous illumination.

Absorption spectra have been normalized at 710 mμ.

time. Subsequent illuminations form more chlorophyll and the prominence of the carotenoid excitation bands increasing during the intervening dark periods (curves E, F, and G).

Discussion

Wolff and Price (1957) and Virgin (1960) showed that the newly formed chlorophyll is not esterified with phytol. Protochlorophyllide, not protochlorophyll, is transformed by light. The phytolization of the chlorophyllide occurs within an hour after its initial formation. Phytolization puts a lipophilic tail on the molecule which can be dissolved in the lipid present in the plastid and bring the chlorophyll into juxtaposition with the lipophilic carotenoid pigments. Resonance transfer can then occur.

Electron micrographs (Hodge, McLean and Mercer, 1956, Muhlethaler and Frey-Wyssling, 1959) show increasing numbers of lamellae and association of lamellae with increasing chlorophyll accumulation. The curves of Figure 1 show that the efficiency of resonance transfer between carotenoid pigments and chlorophyll increases with increasing chlorophyll formation. One would expect the efficiency of transfer to increase with increasing structural development.

The development of resonance transfer depends upon the development of structure and therefore can be used as an assay for structure in a system of developing chloroplasts. Such an assay can be used to determine whether various physical and chemical agents affect plastid development.

References

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