A FAR RED ABSORBING FORM OF CHLOROPHYLL

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The existence of a form of chlorophyll which absorbs light at somewhat longer wavelength than chlorophyll a has been postulated by Duysens (1952), Franck (1955), Kok (1959) and as well as by others. Such a chlorophyll molecule could drain the excitation energy out of several hundred chlorophyll a molecules and serve as a center of photochemical activity. The most direct evidence for a long wavelength form of chlorophyll has been presented by Brody (1958). He showed that at -193° C. a new band appeared in the fluorescence emission spectrum of chlorella which was several fold more intense than the room temperature fluorescence band of chlorophyll a at 685 mμ. The same band appeared in the fluorescence spectrum of concentrated (but not dilute) ethanolic solutions of chlorophyll a at -193° C. He attributed this fluorescence to a chlorophyll dimer (or higher polymer) which was quenched at room temperature. This note concerns the further characterization of the 720 mμ fluorescing component in an effort to determine whether it could serve as the "active center" for photosynthesis. The evidence supports the view that it can serve such a function. The absorption band of this component is at 705 mμ and excitation energy is transferred to it from chlorophyll a.

Methods

The measurement of fluorescence excitation spectra and absorption spectra of bean leaves with a single-beam recording spectrophotometer (Butler and Norris, 1960) has been described previously (Butler, 1960). Fluorescence excitation spectra were measured by placing a 730 mμ cut-off filter (Corning filters No. 2600, 9863, and 5030) between the leaf and the phototube. Exciting light from the scanning monochromator of wavelengths shorter than the cut-off wavelength was incident on the top of the leaf while the phototube viewed the underneath side of the leaf through the cut-off filter and measured fluorescence intensity of wavelengths longer than 730 mμ. Absorption spectra were recorded by removing the cut-off filter and measuring the transmitted light directly. The leaf was mounted between two washers which restricted the area to a diameter of 6 mm. Measurements could be made while the leaf was in a Dewar flask, cooled to the temperature of liquid nitrogen. The absorption spectra have been corrected for the spectral system response of the single-beam spectrophotometer and the excitation spectra have been corrected to equal quantum flux of exciting radiation. The energy of the exciting light varied monotonously from 1 erg/sec/cm^2 at 450 mμ to 40 ergs/sec/cm^2 at 700 mμ.

Results

The fluorescence excitation and absorption spectra of a primary leaf from a 7-day-old, greenhouse-grown bean plant measured at room temperature and at -196° C. are shown in Fig. 1. The fluorescence spectra show very little detail because at all visible wavelengths a large fraction of the incident light is absorbed by chlorophyll a or by accessory pigments which transfer their excitation energy to chlorophyll a. At 25° C. the fluorescence beyond 730 mμ is due primarily to the long wavelength tail of the chlorophyll a fluorescence. Excitation bands are apparent at 680 mμ due to chlorophyll a and at 650 mμ due to chlorophyll b which sensitizes chlorophyll a fluorescence. There is also the hint of an excitation band at about 700 mμ which is much more prominent at low temperature. At -196° C. the intensity of fluorescence of wavelengths longer than 730 mμ increases approximately 20 fold at all exciting wavelengths. This increased fluorescence at low temperature is due to the appearance of the 720 mμ fluorescence band (Brody, 1958). The intensity of the 720 mμ emission at -196° C. is greater

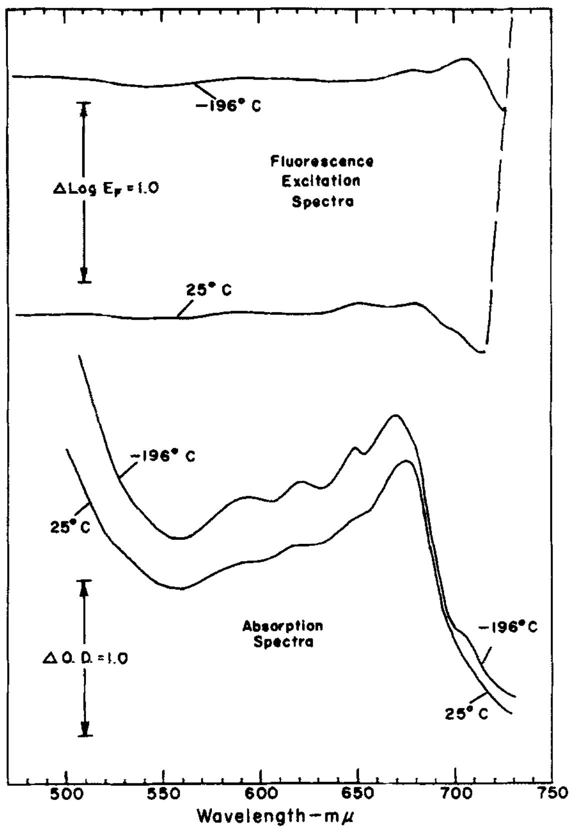

Fig. 1 Fluorescence excitation and absorption spectra of a primary leaf from a 7-day-old, greenhouse-grown bean plant at 25° C. and -196° C. The steep rise of the excitation spectra is due to the transmission of the cut-off filter.

than the 685 mμ fluorescence band of chlorophyll a at room temperature and a much greater proportion of it extends beyond 730 mμ. The main excitation band at -196° C. which is due to the absorption of light by the 720 mμ fluorescing component is at 705 to 710 mμ. The absorption spectrum of the leaf at -196° C. confirms the presence of the 705 mμ absorption band. The increased spectral resolution at -196° C. is required for the detection of the absorption band of this component which presumably is a form of chlorophyll.

Discussion

The low temperature spectra clearly show the existence of a low concentration of a 705 mμ absorbing component which accepts excitation energy from chlorophyll a. At room temperature where the fluorescence of the long wavelength component is quenched, absorption of light by chlorophyll a and the accessory pigments result in the normal chlorophyll a fluorescence peaked at 685 mμ. At low temperature the 720 mμ fluorescence band predominates and the absorption of light by chlorophyll a and the accessory pigments result in the fluorescence emission by the 705 mμ absorbing component. Thus, the main bulk of the chlorophyll a has transferred excitation energy to the long wavelength chlorophyll even though this component is present in much lower concentration. Energy transfer to the long wavelength component as evidenced by sensitized fluorescence requires a low temperature where the fluorescence of this component is not quenched. Data on the quenching of chlorophyll a fluorescence in vivo at room temperature (not included here) indicate that the long wavelength chlorophyll is present and accepts excitation energy from chlorophyll a at room temperature as well. More experimental data and a discussion of the role of the long wavelength chlorophyll in photosynthesis will be presented elsewhere in a full length paper.

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