Short Communications

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Effect of light intensity on the far-red inhibition of chlorophyll *a* fluorescence *in vivo*

The results of a previous paper[1] showed that red light increased the fluorescence yield of chlorophyll *a in vivo* and that far-red light, with a maximal effectiveness at 705 mμ, decreased the yield. The present communication reports on the effects of light intensity on the far-red inhibition of the fluorescence yield.

The methods of measurement were the same as those previously described. Fluorescence from a green leaf was excited by a low-intensity (10 ergs/sec/cm^2), monochromatic beam at 650 mμ. A multiplier phototube with a cut-off filter measured the intensity of fluorescence at wavelengths longer than 730 mμ. The intensity of fluorescence excited by the low-intensity measuring beam was a measure of the fluorescence yield. Changes in the fluorescence yield of chlorophyll *a* were produced by brief irradiations from an actinic monochromator which consisted of two, wavelength-coupled, 200 $\times$ 25 mm wedge interference filters and a 3-mm slit. The filament of a G.E. 200-W quartzline lamp was focused on the slit with an *f*/1.5 lens system. The actinic beam had a pass band of 15 mμ and an intensity of 10000 ergs/sec/cm^2 which was independent of wavelength from 600–800 mμ. The intensity was reduced to 2000 and 600 ergs/sec/cm^2 with neutral density filters. Intensities up to 20000 ergs per sec/cm^2 were obtained by using one wedge interference filter with a glass cut-off filter. Fluorescence was measured immediately after, but not during the actinic irradiation. Measurements were made with the leaf in N_2 because the irradiation-induced fluorescence-yield changes persist longer when the leaf is anaerobic.

The leaf was alternately irradiated for 5 sec with the actinic monochromator set first at 650 mμ and then at a longer wavelength. The intensity of fluorescence

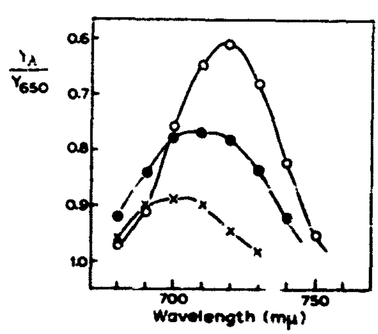

Fig. 1. Action spectra for the far-red inhibition of the enhanced fluorescence yield. The ratio of the fluorescence yield after a 5-sec irradiation at a long wavelength, Y_λ, to the fluorescence yield after a 5-sec irradiation at 650 mμ, Y_{650}, as a function of the wavelength. Intensities of actinic light were: ○—○, 10000 ergs/sec/cm^2; ●—●, 2000 ergs/sec/cm^2; and ×—×, 600 ergs per sec/cm^2.

excited by the low-intensity beam was recorded for about 10 sec after each actinic irradiation to establish the relative fluorescence yield. The actinic irradiation at 650 mμ increased the fluorescence yield of the dark-conditioned leaf and the subsequent irradiation at the longer wavelength reversed the enhanced yield to a degree dependent upon this wavelength. At 10000 ergs/sec/cm^2, 720 mμ was the most effective wavelength in suppressing the yield. At lower intensities, however, as were used in the previous work, the action maximum shifted to shorter wavelength. Action spectra for the far-red inhibition of the red-enhanced fluorescence yield obtained with the same leaf, using actinic intensities of 10000, 2000 and 600 ergs/sec/cm^2, are shown in Fig. 1. With actinic intensities of 20000 ergs/sec/cm^2, action maxima have been obtained as far out as 750 mμ.

The shift of the action maximum with increasing intensity can be explained on the basis of two opposing photoreactions which saturate at different intensities. The red-light effect of increasing the fluorescence yield is presumably mediated by chlorophyll *a* while the far-red effect of suppressing the yield is presumably due to C-705. Light in the 700-mμ region is absorbed by both C-705 and the long-wavelength tail of the chlorophyll *a* absorption band. Fig. 1 shows that, at a wavelength such as 690 mμ, increasing the intensity from 600 to 2000 ergs/sec/cm^2 increased the far-red action but that increasing the intensity further to 10000 ergs/sec/cm^2 results in a decreased action. Apparently, at 690 mμ, the far-red effect begins to saturate between 600 and 2000 ergs/sec/cm^2 while the opposing red effect continues to increase with increasing intensity. Light of longer wavelengths, however, will be less effective in opposing the far-red effect because less of the light is absorbed by chlorophyll *a*. The action spectrum, which measures the net difference between the far-red and red effects, will thus have a longer wavelength maximum at higher intensity.

The significance of the fluorescence-yield changes to photosynthesis is not known. It seems likely, however, that the red and far-red effects of light on the fluorescence yield have the same photochemical basis as the two-wavelength effects of the second Emerson effect. If this is true, the action spectra for other photosynthetic reactions which have maxima in the far-red region may also shift with the intensity of the activating light. Thus, photosynthetic action spectra which show maxima in the region of 710 to 750 mμ do not necessarily involve pigments which have corresponding absorption bands, but may be due to C-705.

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[1] W. L. BUTLER, *Biochim. Biophys. Acta*, 64 (1962) 309.

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