THE EMERSON ENHANCEMENT EFFECT IN TPN-PHOTOREDUCTION BY SPINACH CHLOROPLASTS*

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Emerson and co-workers (1957-1960) observed that the low quantum yield of photosynthesis by far red light (> 680 mμ) can be enhanced by simultaneous exposure to shorter wavelength light which is absorbed by pigments other than chlorophyll a. The existence of this effect has been interpreted to mean that there are two separate photochemical reactions necessary for complete photosynthesis.

R. Govindjee (1960, 1961) reported experiments with the quinone Hill reaction, which showed that the effect also occurred with oxygen evolution in the Hill reaction. However, unpublished data of one of us (R.G.) show lack of enhancement in the ferricyanide Hill reaction. It, therefore, seems that the mechanism of the Hill reaction by different Hill oxidants may not be one and the same. We undertook the present study of enhancement in chloroplast reaction using Triphosphopyridine Nucleotide (TPN) as the oxidan

METHODS

Chloroplasts were prepared from spinach leaves by a method similar to that of Hill and Walker (1959) and were suspended in NaCl-sucrose-tris buffe (pH 7.5). The reaction mixture contained the following ingredients in μmole

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KH_2PO_4:100 (pH7.3), $MgCl_2$:15, ADP:2 and TPN:1. TriphosphoPyridine Nucleotide Reductase was prepared according to the method of San Pietro and Lang (1958) through the Dowex-Bentonite step and was added in optimum quantities. Chloroplasts containing 100 µg of chlorophyll were used. The total volume of the reaction mixture was 2 ml.

Light of different wavelengths (690 mµ to 740 mµ at intervals of 10 mµ) was obtained from tungsten lamps by means of Bausch and Lomb second order interference filters combined with sharp cut-off Schott and Corning filters. Supplementary light was provided from a 40 watt fluorescent tube. The tube did not produce exactly the same intensity of light throughout its length and this is reflected in column 3 of Table II. Six Beckman cuvettes containing reaction mixture were exposed for 10 minutes to different wavelengths of far red light (690 mµ - 740 mµ). An identical set of six cuvettes was exposed to white light alone and a third set to the combined lights. A dark control was always maintained. Temperature of the water bath was maintained at 20°C.

After light exposures, chloroplasts were centrifuged out and the amount of photoreduction of TPN was determined by measuring the optical density at 340 mµ.

RESULTS AND DISCUSSION

Rate of photoreduction of TPN was first measured versus intensity of light to ensure that we were working in the linear range. Table I shows a sample result at 710 mµ.

Table II shows the effect of supplementary white light on the light action of far red light. The Emerson enhancement, which is the ratio of the light action of far red light in presence of supplementary light to the light action of far red light in the absence of supplementary light was calculated as follows:

$$\frac{R_{(\text{far red + white lights})} - R_{(\text{white light})}}{R_{(\text{far red light})}},$$

TABLE I

Effect of Increasing Light Intensity on TPN-Reduction in 710 mμ Light

Intensity in Arbitrary Units	Optical Density at 340 mμ (Corrected for dark control)
14.3	.131
22.0	.210
33.0	.303
65.0	.621
100.0	.905

TABLE II

Effect of Supplementary White Light on TPN-Reduction in Far Red Light

mμ Far Red Light	OPTICAL DENSITY AT 340 mμ (Corrected for dark control)			Emerson Enhancement
	Exposure to Far Red Light	Exposure to White Light	Exposure to Combined Lights	
690	.117	.576	.778	1.73
700	.206	.589	.924	1.66
710	.239	.544	.958	1.73
720	.198	.598	1.002	2.04
730	.144	.512	.840	2.28
740	.086	.570	.840	3.14

where R stands for optical density measured at 340 mμ that has been correcte for dark control.

The calculated ratios are shown in column 5 of Table II.

We have repeated the experiment shown in Table II four times and have obtained qualitatively similar results. The data presented here is an average result of all the four experiments. We have also confirmed this

enhancement in the photoreduction of TPN by measuring the concomitant oxygen evolution with a platinum electrode.

Our results (shown in Table II) clearly show the existence of Emerson enhancement effect in TPN photoreduction in spinach chloroplasts. We interpret this to mean that the TPN-Hill reaction requires two photochemical steps.#

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#While this paper was being written, our attention was drawn to two abstracts in Plant Physiology, 37, (Supplement) (1) S. A. Gordon, "Observations in TPN Photoreduction in Preparations of Laurencia obtusa," p. V, and (2) B. C. Mayne and A. H. Brown, "A Comparison of the Emerson Two-Light Effect in Photosynthesis and the Hill Reaction," p. LXV.