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

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Received August 29, 1962

Emerson and co-workers (1957-1960) observed that the low quantum yield of photosynthesis by far red light (> 680 mp) 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 However, unpublished data of one of us (R.G.) show lack of enhancement in the ferricyanide Hill reaction.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 oxidant.

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 buffer (pH 7.5). The reaction mixture contained the following ingredients in μmol:

* This investigation was supported by National Science Foundation², U. S. Public Health Service¹, and the Air Force Office of Scientific Research³.
KH$_2$PO$_4$:100 (pH 7.3), 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 μm to 740 μm at intervals of 10 μm) 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 μm - 740 μm). 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 μm.

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 μm.

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:

$$R(\text{far red + white lights}) - R(\text{white light}) \over R(\text{far red light}),$$

223
TABLE I
Effect of Increasing Light Intensity on TPN-Reduction in 710 μm Light

<table>
<thead>
<tr>
<th>Intensity in Arbitrary Units</th>
<th>Optical Density at 340 μm (Corrected for dark control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.3</td>
<td>.131</td>
</tr>
<tr>
<td>22.0</td>
<td>.210</td>
</tr>
<tr>
<td>33.0</td>
<td>.303</td>
</tr>
<tr>
<td>65.0</td>
<td>.621</td>
</tr>
<tr>
<td>100.0</td>
<td>.905</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Energy of Light in μm</th>
<th>Exposure to Far Red Light</th>
<th>Exposure to White Light</th>
<th>Exposure to Combined Lights</th>
<th>Emerson Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>690</td>
<td>.117</td>
<td>.576</td>
<td>.778</td>
<td>1.73</td>
</tr>
<tr>
<td>700</td>
<td>.206</td>
<td>.589</td>
<td>.924</td>
<td>1.66</td>
</tr>
<tr>
<td>710</td>
<td>.239</td>
<td>.544</td>
<td>.958</td>
<td>1.73</td>
</tr>
<tr>
<td>720</td>
<td>.198</td>
<td>.598</td>
<td>1.002</td>
<td>2.04</td>
</tr>
<tr>
<td>730</td>
<td>.144</td>
<td>.512</td>
<td>.840</td>
<td>2.28</td>
</tr>
<tr>
<td>740</td>
<td>.086</td>
<td>.570</td>
<td>.840</td>
<td>3.14</td>
</tr>
</tbody>
</table>

where R stands for optical density measured at 340 μm that has been corrected 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. #

ACKNOWLEDGMENTS

Thanks are due to Miss Iris Martin for her help in preparing the enzyme and to Dr. Bessel Kok for reading the manuscript.

REFERENCES


#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.