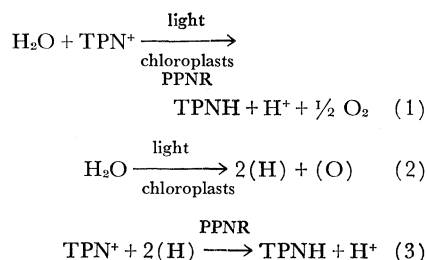


Action Spectrum for Triphosphopyridine Nucleotide Reduction by Illuminated Chloroplasts

In the presence of the enzyme, photosynthetic pyridine nucleotide reductase (PPNR), chloroplasts carry out a light-dependent reduction of triphosphopyridine nucleotide (TPN) with a concomitant evolution of oxygen (1). The over-all process (Eq. 1) appears to require at least two component systems—namely, a photolytic system for the generation of reducing and oxidizing potential represented by (H) and (O), respectively (Eq. 2), and a system which can utilize the reducing potential formed by the photolysis of water for the reduction of TPN to yield reduced TPN (TPNH) as indicated in Eq. 3. Under these conditions, the oxidizing potential is ultimately released as molecular oxygen.



The evidence available favors the hypothesis that the reaction depicted by Eq. 2 is the primary photochemical act not only in the photosynthetic reduction of TPN, but also in photosynthesis, the Hill reaction, and photosynthetic phosphorylation. According to this formulation, the action spectrum for the reduction of TPN by illuminated chloroplasts should be identical with that for photosynthesis, the Hill reaction, and photosynthetic phosphorylation. Indeed, the action spectra for the latter three processes have been shown previously to correspond both to one another and to the absorption spectrum of chlorophyll (2). In this report (3), data are presented which indicate that the action spectrum for the reduction of TPN by illuminated chloroplasts also corresponds to the absorption spectrum of chlorophyll.

The experiments were performed with the high irradiance spectrograph of the U.S. Department of Agriculture at Beltsville, Maryland, as follows: Each reaction mixture contained chloroplasts (4) equivalent to 75 μg of chlorophyll, 1 μmole of TPN, 185 μg of partially purified PPNR prepared as described by San Pietro and Lang (5) and 265 μmole of tris buffer, pH 7.2, in a final volume

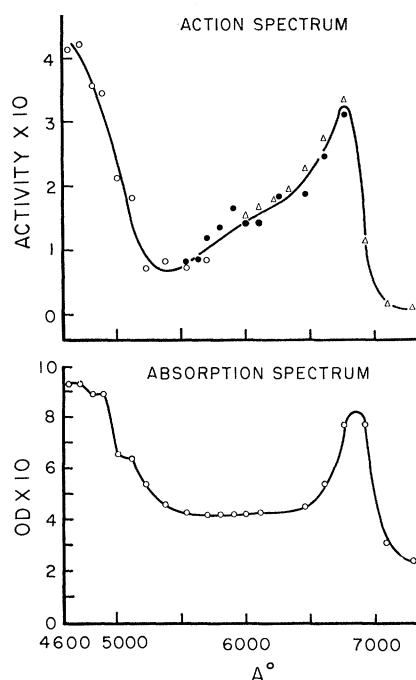


Fig. 1. (Top) Action spectrum for TPN reduction by illuminated chloroplasts. The activity is expressed as micromoles of TPN reduced per average incident micro-Einstein per reaction mixture per 10 minutes. The different symbols signify three separate experiments. (Bottom) Absorption spectrum of the reaction mixture diluted threefold.

of 3 ml. The optical density of each reaction mixture at 340 $\text{m}\mu$ was measured before and after illumination for 10 minutes at room temperature with light of different wavelengths. The increase in optical density calculated from these two measurements serves as a measure of TPN reduction.

All optical density measurements were made with a Beckman spectrophotometer, model DU, with photomultiplier attachment; a complete reaction mixture which was kept in the dark at room temperature was used as a blank. The amount of energy available at each wavelength tested was determined with a thermopile.

The action spectrum for this process is presented in Fig. 1. This action spectrum was obtained by correcting the observed increase in optical density at 340 $\text{m}\mu$ for the amount of energy available at each wavelength tested (6). It is apparent from Fig. 1 that red light and blue light are most effective for the reduction of TPN by illuminated chloroplasts and that green light is least effective. Within the spectral range measured (4630 to 7290 \AA) the action spectrum for this

process closely parallels the absorption of the chloroplasts, as is indicated in the lower portion of Fig. 1, and that of chlorophyll.

It can be calculated that at 6720 \AA and an incident intensity of 2.88×10^3 $\text{erg/cm}^2 \text{ sec}$, the quantum requirement for the reduction of TPN is approximately 16 quanta per molecule of TPN reduced (or atom of oxygen evolved). This value agrees very closely with the value of 17 quanta per atom of oxygen evolved in the Hill reaction, with ferricyanide as the electron acceptor, reported by Lumry *et al.* (7).

In conclusion, the action spectrum for the reduction of TPN by illuminated chloroplasts has been shown to resemble closely the absorption spectrum of the chloroplast suspension and the action spectra for the Hill reaction, photosynthesis, and photosynthetic phosphorylation. This finding supports the hypothesis that all four processes require the same primary photochemical act—namely, the photolysis of water.

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References and Notes

1. A. San Pietro and H. M. Lang, *Science* **124**, 118 (1956); D. I. Arnon, F. R. Whately, M. B. Allen, *Nature* **180**, 182 (1957).
2. A. T. Jagendorf, S. B. Hendricks, M. Avron, M. B. Evans, *Plant Physiol.* **33**, 1 (1958); S. L. Chen, *ibid.* **27**, 35 (1952).
3. This report is contribution No. 236 of the McCollum-Pratt Institute. This investigation was supported in part by a research grant (RG-4143 -C3-) from the National Institutes of Health, U.S. Public Health Service.
4. The chloroplasts were prepared as follows: 25 g of spinach were depeetioled and ground in a mortar and pestle, in the cold, with about 4 g of sand and 35 ml of 0.05M $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ buffer, pH 7, containing 0.4M sucrose and 0.01M KCl. The homogenate was filtered through cheese cloth and the filtrate was centrifuged for 1 minute at 200g in the cold. The supernatant was then centrifuged for 7½ minutes at 2500g. The final residue was made up in 13 ml of the 0.05M $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ buffer, pH 7, containing 0.4M sucrose and 0.01M KCl.
5. A. San Pietro and H. M. Lang, *J. Biol. Chem.* **231**, 211 (1958).
6. Due to the high absorption of the reaction mixtures used in these experiments, the average incident light intensity for each reaction mixture was used to determine the action spectrum. The average incident light intensity equals

$$\frac{I_0 [1 - (I/I_0)]}{2.3 \log \text{O.D.}}$$

where I_0 is the incident intensity, I is the intensity of emerging light, and O.D. is the optical density of the reaction mixture.

7. R. Lumry, R. E. Wayrynen, J. D. Spikes, *Arch. Biochem. Biophys.* **67**, 453 (1957).

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