Studies on Photosynthetic Processes

II. ACTION SPECTRA AND QUANTUM REQUIREMENT FOR TRIPHOSPHOPYRIDINE NUCLEOTIDE REDUCTION AND THE FORMATION OF ADENOSINE TRIPHOSPHATE BY SPINACH CHLOROPLASTS

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Wave length dependency of photosynthesis in intact tissues has been examined frequently in studies of the photosynthetic mechanism. In most such studies, oxygen production was determined as a function of wave length, or difference spectra were observed in efforts to elucidate this process. With the demonstration of light-induced adenosine triphosphate synthesis associated with reduced triphosphopyridine nucleotide formation in isolated chloroplasts (1, 2), it became experimentally feasible to examine the influence of light on the formation of these two compounds, which are considered to be the “driving force” of the CO₂ photosynthesis cycle. It is generally believed that the photochemical act produces ATP and TPNH in a molar ratio of unity (3, 4). In Paper I of this series (5), it was observed that the ratio of ATP to TPNH in white light approaches the value of 1 only at saturating light intensities. The ratio was observed to drop sharply as the light intensity was lowered, reaching a value of 0.03 at 10 foot-candles. Since it has been postulated that ATP formation is “coupled” to the reduction of TPN(3), and since the ratio was unity only at saturating levels of white light, it became necessary to examine the response of the system in monochromatic light. In this communication, we describe the effect of monochromatic light on TPN reduction and the associated formation of ATP in the spinach chloroplast. Data are also presented on the effect of monochromatic light on the synthesis of ATP by the spinach chloroplast in the presence of phenazine methosulfate.

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EXPERIMENTAL PROCEDURE

Materials—TPN, ADP, and PMS were obtained from the Sigma Chemical Company. PPNR was purified from spinach leaves through the Dowex-bentonite step described by San Pietro (6). This preparation had a specific activity of 22 units per mg of protein. P₃₂ was purchased from the Squibb Laboratories and treated with HC₁₂O₅ before use. Preparation of chloroplast fragments from spinach was described previously (5).

Reaction Conditions and Methods of Analysis—The reaction mixtures for the assay with TPN contained the following components: Tris, pH 7.5, 20 μmoles; MgCl₂, 8 μmoles; TPN, 1.5 μmoles; ADP, 2.5 μmoles; KH₂PO₄-K₂HPO₄, pH 7.5, 1.54 μmoles (containing approximately 1 μc of P₃₂); chloroplast fragments containing 21 to 26 μg of chlorophyll; PPNR, 0.22 unit of the purified preparation; total volume, 2 ml. For assay with PMS, all components were the same except that 0.25 μmole of PMS was substituted for TPN and PPNR. Both TPN and PMS reaction mixtures were illuminated simultaneously with the same chloroplast preparations so that experimental differences might be minimized in obtaining comparable data.

After irradiation at room temperature (22°), the TPN containing mixtures were centrifuged and aliquots of the supernatant fluid were analyzed for TPNH and ATP₂ as described elsewhere (5). Phosphorylation supported by PMS was stopped by adding 0.3 ml of 20% trichloroacetic acid to the reaction mixture. After centrifugation, a sample of the supernatant solution was assayed for ATP₂. Chlorophyll was determined by the method of Arnon (8).

Irradiation—The Argonne Biological grating spectrograph (9) was used as the radiation source. Incident energies were determined with an Epple thermopile (calibrated to a standard lamp) and Liston-Becker amplifier and corrected for reflection loss at the front surface of the sample cells. Energy absorbed at each wave length was approximated as the difference between incident and exit energies, I₀ - I. The exit energy was calculated from the relationship A = -log₁₀(I/I₀), in which A is the absorbancy

The abbreviations used are: TPN, triphosphopyridine nucleotide; PMS, phenazine methosulfate; PPNR, photosynthetic pyridine nucleotide reductase.
taken from the absorption spectrum of an identical preparation. The energy absorbed in joules was converted to Einstein units to determine the quantum requirement per mole of TPNH or ATP at each wave length. The calculated yields may be considered as conservative estimates of efficiencies since no correction was made for energy loss by scatter. Reaction mixtures were irradiated laterally in quartz or Pyrex cuvettes 1 cm in width and depth. For this width, well over 95% of the incident energy may be presumed to fall within 4 mp about each wave length locus (10).

RESULTS AND DISCUSSION

Absorption Spectra—The absorption spectrum of the TPN-containing reaction solution is given in Fig. 1. It shows the characteristic major red and blue bands of chlorophyll a and b. The shoulder at 480 mp is generally considered to indicate carotenoids (11). The reaction mixture containing PMS has a similar absorption spectrum with an additional peak at about 388 mp.

Effect of Irradiation Intensity—TPN reduction was studied in preliminary experiments to determine the range where the reaction was proportional to irradiation intensity. In Fig. 2, the reduction of TPN at 675 mp is linear to about 100 kiloergs per mm² of incident energy. In similar experiments at 450 mp, the same relation was observed. Therefore, 100 kiloergs per mm² was the maximal incident energy used at any wave length in the subsequent experiments. The assumption has been made that ATP formation associated with TPN reduction is also linear, since ATP formation is saturated in white light at the same intensity as TPN reduction (5). ATP formation in the presence of PMS is also assumed to be linear (7).

Action Spectra of TPN Reduction and ATP Formation——The action spectrum of TPN reduction is given in Fig. 3. This spectrum has peaks at 450, 500, and 675 mp, a shoulder from about 375 to 430 mp, and troughs at 480 mp and from 525 to about 600 mp. The action spectrum of ATP associated with TPN reduction has two peaks, one at 430 mp and another at 680 mp, with shoulders at 375 to 420 mp and at 500 mp, and a trough from 525 to about 600 mp (Fig. 4). The peak at 500 mp observed with TPN reduction is quite distinct and contrasts to the shoulder at 500 mp in the action spectrum of ATP formation in this system. With peaks at 430, 500, and 680 mp, the action spectrum of phosphorylation supported by PMS resembles the phosphorylation “coupled” to TPNH formation, except that the peaks are sharper (Fig. 5).

The action spectrum for TPN reduction with spinach chloroplasts has also been examined by San Pietro et al. (12). Within the spectral range measured by them (463 to 729 mp), the action spectrum of TPN reduction illustrated in Fig. 3 is similar to that given in their publication with the exception of the peak observed by us at 500 mp.

Action spectra of photophosphorylation by spinach chloroplasts in the presence of PMS have been reported by two groups.
The curve given in a preliminary note by Jagendorf et al. (13) shows a peak at about 675 μm, in agreement with the data recorded here. In contrast, the action spectrum published by Kök and Hoch (14) shows a peak at 710 μm and a trough at about 665 μm. We have examined the wave lengths about 700 μm in detail, and could not find any indication of a peak in that region. All of the processes studied showed decreased effectiveness above 700 μm, with very high quantum requirements.

Comparison of Action Spectrum and Absorption Spectrum—It may be noted that the action spectra are similar to the absorption spectra except in the region generally attributable to carotenoids. Whereas the absorption spectra exhibit a shoulder at about 480 μm, the action spectra show a distinct enhancement at about 500 μm which functions in TPN reduction and photophosphorylation. It is probable that this represents absorption of light quanta by carotenoid pigments and a channeling of this energy into a pathway giving rise to the photosynthetic reductant. This finding is in agreement with the data of Fuller et al. (15), who have recently reported that light absorbed by Chromatium carotenoids is able to catalyze PMS-supported photophosphorylation.

Quantum Requirements—Both TPN reduction and ATP formation exhibit highest quantum efficiencies in the red region. The ratio of the lowest quantum requirement in the blue region to that in the red is 1.4 in both instances. The required quanta per molecule of ATP formed in the presence of PMS is about 10 to 15 times greater than ATP formation during TPN reduction. If a light-induced phosphorylation similar to that supported by PMS and fragmented chloroplasts occurs in the intact cell, one may question its physiological value since photophosphorylation in vitro associated with TPN reduction is a much more efficient process. The lowest quantum requirement with PMS, observed only once, was 97 quanta per molecule of ATP at 675 μm. A requirement of 180 quanta per molecule of ATP was the usual value observed with PMS at 675 μm, whereas the average quanta required for TPN supported phosphorylation was 15 at the same wave length. This quantum value of 15 for phosphorylation contrasts with 9 for concomitant reduction of TPN or 18 per molecule of oxygen evolved. At wave length 672 μm, San Pietro et al. (12) obtained a quantum requirement of 16 for the reduction of TPN.

In intact tissues, the quantum efficiency has been shown to be fairly independent of wave length throughout the visible spectrum from 400 to about 650 μm (10). According to the data plotted in Figs. 3, 4, and 5, the particulate preparation showed a quantum efficiency generally independent of wave length in the blue (400 to 525 μm) and red (625 to 650 μm) portions of the spectrum but a dependence on wave length between 525 and 625 μm. Whether this decreased efficiency in TPNH and ATP yield during illumination with photons of 525 to 625 μm is
due to some of the pigments being inactivated during isolation or to a generally lower efficiency of energy transfer by some pigments in the isolated particle is not known. Fuller et al. (15) have observed that the ability of the carotenoids to transfer energy to bacteriochlorophyll for photophosphorylation by *Chromatium* particles depends upon the composition of the isolation medium.

**Ratio of ATP to TPNH**—In Fig. 6, the molar ratio of ATP formed to TPN reduced is given for various wave lengths. It should be noted that the upper curve was plotted with data obtained with irradiation intensities 8 times those used for the lower curve. A ratio of 1 was approached only in the experiments with the higher intensities. These results with monochromatic light further support the observation that the ratio of ATP to TPNH is unity only at saturating light intensities (5). The highest ratios were obtained in the blue region; in contrast, the highest quantum yields were in the red region for both the phosphorylation and reduction.

The possibility should be considered that TPN reduction and phosphorylation are not directly “coupled” processes. Alternatively, these reactions may result from the interaction of different photochemical products formed by the action of light on different pigment complexes. Consistent with this speculation are the following observations. The action spectra show small differences in the peaks for phosphorylation and TPN reduction, the quantum requirements are not similar, and the molar ratio of ATP to TPNH is unity only at saturating light intensities.

**SUMMARY**

Action spectra and quantum requirements were determined with spinach chloroplast fragments for the processes of triphosphopyridine nucleotide reduction, adenosine triphosphate formed during this reduction, and adenosine triphosphate formation supported by phenazine methosulfate. The action spectrum for triphosphopyridine nucleotide reduction showed peaks at 450, 500, and 675 m\(\mu\); peaks for concomitant phosphorylation were at 430 and 680 m\(\mu\), with a shoulder at 500 m\(\mu\). Phosphorylation in the presence of phenazine methosulfate exhibited peaks at 430, 500, and 680 m\(\mu\). Carotenoid pigments (500-m\(\mu\) absorption band) appeared to function in the processes studied. The quantum requirement for triphosphopyridine nucleotide reduction was 9 at 675 m\(\mu\), and for the associated phosphorylation was 15, but the phosphorylation supported by phenazine methosulfate had a quantum requirement from 10 to 15 times greater. The molar ratio of adenosine triphosphate to reduced triphosphopyridine nucleotide only approached one at high light intensities. The highest ratios were obtained in the blue absorption bands; in contrast, the highest quantum yields were in the red region for both phosphorylation and reduction.

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