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 compounds, which are considered to be the “driving force” for the synthesis of ATP by the spinach chloroplast in the presence of phenazine methosulfate.

The photochemical act produces ATP and TPNH in a molar ratio of unity in isolated chloroplasts (1, 2) it became experimentally feasible to examine the influence of light on the formation of these two compounds. Demonstration of light-induced adenosine triphosphate synthesis is generally considered to be the “driving force” of the CO₂ photosynthesis cycle. It is generally believed that compounds, which are considered to be the “driving force” for the synthesis of ATP by the spinach chloroplast in the presence of phenazine methosulfate.

The action spectra and quantum requirements for triphosphopyridine nucleotide reduction and the formation of adenosine triphosphate by spinach chloroplasts are also presented on the effect of monochromatic light on 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.

EXPERIMENTAL PROCEDURE

Materials—TPN, ADP, and PMS² 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 n HCl and charcoal (7) 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 7.8, 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 10 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 purified preparation 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 EppleJ 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: PMS, phenazine methosulfate; PPNR, photosynthetic pyridine nucleotide reductase.

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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 ir-
radiated 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 μm about each wave length locus

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 μm is generally considered to indicate
carotenoids (11). The reaction mixture containing PMS has a
similar absorption spectrum with an additional peak at about
388 μm.

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 μm is linear to about 100 kiloergs per
mm² of incident energy. In similar experiments at 450 μm, 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 μm, a shoulder from
about 375 to 430 μm, and troughs at 480 μm and from 525 to
about 600 μm. The action spectrum of ATP associated with
TPN reduction has two peaks, one at 430 μm and another at 680
μm, with shoulders at 375 to 420 μm and at 500 μm, and a trough
from 525 to about 600 μm (Fig. 4). The peak at 500 μm ob-
served with TPN reduction is quite distinct and contrasts to the
shoulder at 500 μm in the action spectrum of ATP formation in
this system. With peaks at 430, 500, and 680 μm, 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 chloro-
plasts has also been examined by San Pietro et al. (12). Within
the spectral range measured by them (463 to 729 μm), the action
spectrum of TPN reduction illustrated in Fig. 3 is similar to that
given in their publication with the exception of the peak ob-
served by them at 450 μm.

Action spectra of photophosphorylation by spinach chloro-
plasts in the presence of PMS have been reported by two groups.
Fig. 4. Action spectrum and quantum requirement for ATP formation during TPN reduction. Action spectrum is calculated in micromoles of ATP per kiloerg X 10^2 (■—■■), and quantum requirement is calculated as the quanta per molecule of ATP (○—○○).

Fig. 5. Action spectrum and quantum requirement for phosphorylation supported by PMS. Action spectrum is calculated in micromoles of ATP per kiloerg X 10^2 (■—■■), and quantum requirement is calculated as the quanta per molecule of ATP (○—○○).

The curve given in a preliminary note by Jagendorf et al. (13) shows a peak at about 675 mp, in agreement with the data recorded here. In contrast, the action spectrum published by Kok and Hoch (14) shows a peak at 710 mp and a trough at about 665 mp. We have examined the wave lengths about 700 mp in detail, and could not find any indication of a peak in that region. All of the processes studied showed decreased effectiveness above 700 mp, 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 mp, the action spectra show a distinct enhancement at about 500 mp 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 mp. A requirement of 180 quanta per molecule of ATP was the usual value observed with PMS at 675 mp, 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 mp, 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 690 mp (16). 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 mp) and red (625 to 680 mp) portions of the spectrum but a dependence on wave length between 525 and 620 mp. Whether this decreased efficiency in TPNH and ATP yield during illumination with photons of 525 to 625 mp 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 wavelengths. 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 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 μm; peaks for concomitant phosphorylation were at 430 and 680 μm, with a shoulder at 500 μm. Phosphorylation in the presence of phenazine methosulfate exhibited peaks at 430, 500, and 680 μm. Carotenoid pigments (500-μm absorption band) appeared to function in the processes studied. The quantum requirement for triphosphopyridine nucleotide reduction was 9 at 675 μm, and for the associated phosphorylation was 13, 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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**REFERENCES**

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