Studies on Photosynthetic Processes

I. THE EFFECT OF LIGHT INTENSITY ON TRIPHOSPHOPYRIDINE NUCLEOTIDE REDUCTION, ADENOSINE TRIPHOSPHATE FORMATION, AND CARBON DIOXIDE ASSIMILATION IN SPINACH CHLOROPLASTS*

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Although there is a considerable amount of information on the effect of light intensity on O2 evolution and, to a lesser extent, CO2 assimilation in intact plants (1), there are few reports on the effect of light intensity on the photosynthetic processes known to occur in the isolated chloroplast and chloroplast fragments. Isolated chloroplasts have the ability to assimilate CO2 (2, 3), and chloroplast fragments reduce triphosphopyridine nucleotide and generate adenosine triphosphate from adenosine diphosphate and inorganic orthophosphate when illuminated (4).

Jagendorf and Avron (5) have presented data on the rates of phosphorylation (using phenazine methosulfate or flavin mononucleotide) and the Hill reaction (with trichlorophenol indophenol) as functions of light intensity. Krogmann (6) investigated the effect of light intensity on oxidative phosphorylation by spinach chloroplasts using trichlorophenol indophenol as acceptor. The reduction of diphosphopyridine nucleotide by chromatophores from Rhodospirillum rubrum in various light intensities has also been studied (7). There is, however, no report of the effect of light intensity on adenosine triphosphate formation with triphosphopyridine nucleotide as electron acceptor or on the process of triphosphopyridine nucleotide reduction. Similarly, there is no information on the effect of light intensity on CO2 fixation by chloroplasts. In the present investigation, the effect of light intensity on CO2 fixation by the isolated intact chloroplast and on reduced triphosphopyridine nucleotide and adenosine triphosphate formation by chloroplast fragments were studied.

EXPERIMENTAL PROCEDURE

Reagents—TPN and ADP were obtained from the Sigma Chemical Company. Photosynthetic pyridine nucleotide reductase was prepared from spinach leaves and purified by the method of San Pietro (8). The purified enzyme had a specific activity of 22 units per mg of protein.

Preparation of Chloroplasts—Chloroplasts were prepared from spinach leaves as described previously (9, 10), and the chlorophyll content was determined by the method of Arnon (11). For study of CO2 fixation, the chloroplasts were prepared in a mixture of 0.35 M NaCl and 0.02 M Tris, pH 7.5, and resuspended in this solution after centrifugation. For the assay of TPN reduction and ATP formation, the chloroplasts were prepared in the same way but after centrifugation were suspended in a mixture of 0.055 M NaCl and 0.002 M Tris, pH 7.5, recentrifuged, and suspended in the same medium. The latter preparation consisted essentially of chloroplast fragments.

CO2 Assimilation—For the assay of CO2 fixation the following components were mixed in a Beckman cuvette: Tris, pH 7.5, 80 μmoles; MnCl2, 1 μmole; sodium ascorbate, pH 7.5, 1 μmole; KH2PO4-K2HPO4, pH 7.5, 0.3 μmole; NaCl, 380 μmoles; NaHCO3, 0.29 μmole (containing 3.55 μC of 14CO2); intact chloroplasts (containing approximately 200 μg of chlorophyll); total volume, 1.5 ml. The reaction mixtures were illuminated for 10 minutes with a 200-watt tungsten lamp immersed in a beaker containing 1% (weight per volume) CuSO4·5H2O, and the light intensity was varied by adjusting the distance between the light source and the cuvettes containing the reaction mixtures. Light intensity was measured with a Weston foot-candle meter. The reaction was stopped by the addition of 0.4 ml of 1 N HCl, and samples of 100 μl were taken for the determination of radioactivity (9). Replicate reaction mixtures were incubated in the absence of light for 10 minutes to obtain values for dark CO2 fixation. The temperature in the reaction vessels was 18–20°, and assays of CO2 fixation were carried out as soon as possible after the preparation of the chloroplasts (12).

TPN and ATP Formation—According to the nature of the experiment being performed, two different types of reaction mixtures were used for studying TPN reduction and ATP formation, one for reaction mixtures of low chlorophyll content and one for mixtures of high chlorophyll content. The reaction mixtures for the assay with low chlorophyll content 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, approximately 1.4 μmoles (containing approximately 1 μC of 32P); chloroplast fragments (containing approximately 30 μg of chlorophyll; photosynthetic pyridine nucleotide reductase, 0.22 unit of the purified preparation; total volume, 2 ml. For the assay with high chlorophyll content, the reaction mixtures contained: Tris, pH 7.5, 80 μmoles; MgCl2, 4 μmoles; TPN, 1.5 μmoles; ADP, 2.5 μmoles; KH2PO4-K2HPO4, pH 7.5, approximately 1.4 μmoles (containing approximately 1 μC of 32P); chloro-
RESULTS

Effect of Light Intensity on CO₂ Fixation—Increasing light intensity produced a higher rate of CO₂ fixation but at low light intensities there was a lag in the rate of CO₂ fixation. The extent of this lag was somewhat variable and Fig. 1 shows the results of two experiments. In some experiments light intensity approached saturation values at 3000 foot-candles but in others (Fig. 1, lower curve) CO₂ assimilation was still increasing at this intensity.

Effect of Light Intensity on TPNH and ATP Formation—At low chlorophyll concentrations, the rate of TPN reduction increased steadily with increasing light intensity until saturation values were reached (Fig. 2). ATP formation showed a lag at 10 and 25 foot-candles, and the rate of formation increased thereafter.

With higher chlorophyll concentration (Fig. 3), the rate of TPNH formation again increased linearly with increasing light intensity until light was no longer the limiting factor, but the lag in ATP formation was much more pronounced than in the low
chlorophyll experiments. There was no detectable ATP formation at intensities of 250 foot-candles or less, and the rate lagged until 500 foot-candles. The curves for TPNH production and ATP formation were quite different in form, and the yield of ATP never approached that of TPNH even at the highest light intensity used (3000 foot-candles).

With low chlorophyll reaction mixtures the molar ratio ATP:TPNH approached 1 at 3000 foot-candles and there was a marked fall in the ratio below approximately 250 foot-candles (Fig. 4). ATP:TPNH was 0.50, 0.25, and 0.03 at 50, 25, and 10 foot-candles, respectively. With high chlorophyll reaction mixtures the ratio reached a minimum of 0.5 at 3000 foot-candles and was zero at 250 foot-candles or less.

**DISCUSSION**

This investigation has shown that there are differences in the response of the photosynthetic processes to variations in light intensity. The reduction of TPN was linear with low light intensities, whereas both CO₂ fixation and ATP production lagged in low light. The lag in ATP production was especially pronounced in reaction mixtures with high chlorophyll content comparable to the concentration used in the CO₂ fixation experiments. It is believed that 3 molecules of ATP are required for each molecule of CO₂ reduced (15); the curves for CO₂ fixation and ATP formation in the high chlorophyll reaction mixtures are similar in form, and it is possible that the supply of ATP is a factor limiting CO₂ fixation in the chloroplast at low light intensities. It is unlikely that CO₂ fixation was limited by the formation of TPNH since TPNH production was linear at low light intensities.

The apparent uncoupling of ATP formation and TPN reduction at low light intensities and particularly in reaction mixtures of high chlorophyll content may or may not be a real phenomenon. The absence of linearity between light intensity and phosphate esterification with high chlorophyll concentration is not limited to the case in which TPN is the electron acceptor. Krogmann (6), studying oxidative photophosphorylation with trichlorophenol indophenol as cofactor and a chlorophyll concentration limited to the case in which TPN is the electron acceptor.

A possible explanation could be based on the presence in the chloroplast preparations of an ATPase with a very low Michaelis-Menten constant. Experiments in which ATP and P₄ were incubated with spinach chloroplast fragments in light as well as in dark were performed, but there was no detectable incorporation of radioactivity in ATP. Thus, ATPase activity was not demonstrable. This result agreed with the findings of Avron and Jagendorf (16) and Avron (14), who did not detect ATPase activity in spinach chloroplasts and in Swiss chard chloroplasts, respectively.

The lag in ATP production at low light intensities could be an explanation for some observations on CO₂ fixation and O₂ evolution by spinach chloroplasts. Ryther (17) reported that the marine flagellate *Dunaliella euglena* was unable to assimilate CO₂ at low light intensities even though there was considerable photosynthesis as measured by O₂ production. In an experiment with *Chlorella pyrenoidosa*, Steeman-Nielsen (18) found that at 300 lux, a light intensity where photosynthesis was low compared to respiration, CO₂ assimilation was only one-third that of O₂ evolution.

**SUMMARY**

The effect of light intensity on CO₂ fixation, triphosphopyridine nucleotide reduction and adenosine triphosphate formation by spinach chloroplasts was investigated. Increasing light intensity brought about a higher rate of CO₂ fixation but at low light intensities there was a lag in the rate. At low chlorophyll concentrations (approximately 15 µg per ml) as well as at high chlorophyll concentrations (approximately 120 µg per ml) the rate of triphosphopyridine nucleotide reduction increased linearly with increasing light intensity. In contrast, adenosine triphosphate formation showed a distinct lag at intensities of 25 foot-candles or less at the low chlorophyll concentration and a more pronounced lag up to 250 foot-candles at higher chlorophyll concentrations. With low chlorophyll reaction mixtures, the molar ratio of adenosine triphosphate to reduced triphosphopyridine nucleotide approached unity at 3000 foot-candles. The ratio reached a maximum of 0.5 at 3000 foot-candles and high chlorophyll concentrations. No exchange reaction between inorganic phosphate and adenosine triphosphate was observed with these preparations.

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