Lawrence Berkeley National Laboratory

Recent Work

Title

PIGMENT SYSTEMS AND ELECTRON TRANSPORT IN CHLOROPLASTS I. QUANTUM REQUIREMENTS FOR THE TWO LIGHT REACTIONS IN SPINACH CHLOROPLASTS

Permalink

<https://escholarship.org/uc/item/5mh3j5hf>

Authors

Sun, Alexander
Sauer, Kenneth.

Publication Date

1970-12-01

RECEIVED
DOCUMENTS SECTION

PIGMENT SYSTEMS AND ELECTRON TRANSPORT
IN CHLOROPLASTS I. QUANTUM REQUIREMENTS
FOR THE TWO LIGHT REACTIONS
IN SPINACH CHLOROPLASTS

Alexander Sun and Kenneth Sauer

December 1970

AEC Contract No. W-7405-eng-48

TWO-WEEK LOAN COPY

This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. Division, Ext. 5545

48
LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

UCRL-20227

c.2

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

PIGMENT SYSTEMS AND ELECTRON TRANSPORT IN CHLOROPLASTS

I. QUANTUM REQUIREMENTS FOR THE TWO LIGHT REACTIONS
IN SPINACH CHLOROPLASTS

By Alexander Sun and Kenneth Sauer

Laboratory of Chemical Biodynamics, Lawrence Radiation Laboratory,
and Department of Chemistry, University of California, Berkeley,
California 94720

SUMMARY

From studies of electron-transport reactions of isolated spinach chloroplasts, we observe the following quantum requirements: (A) For the photoreduction of NADP, measured both aerobically and anaerobically, in a 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) poisoned system with ascorbate and reduced 2,6-dichlorophenolindophenol (DPIP_H) present as electron donors, the quantum requirements are 1.0 ± 0.05 at wavelengths longer than 700 nm of actinic light, and 1.5 to 2.5 for wavelengths between 620 and 680 nm. (B) For the photoreduction of DPIP with water as the electron donor, the quantum requirements are 1.0 ± 0.05 in the range 630 to 660 nm. (C) For the photoreduction of NADP with water as the electron donor, the quantum requirements are 2.0 ± 0.1 in the wavelength range 640 to 678 nm of actinic light, increasing to 6 or greater at wavelengths beyond 700 nm. These results are shown to be inconsistent with the "separate package" model for the two pigment systems in higher plant photosynthetic electron transport. The evidence is most easily interpreted using a "controlled spillover" model, in which the transfer of electronic excitation energy from one pigment system to the other is under the control of incompletely identified factors in the reaction mixture.

At moderate light intensities the steady state rate of the [ascorbate + DPIP_H $\rightarrow$ NADP] reaction (A) in the presence of DCMU and added ferredoxin can be increased more than three times when saturating amounts of plastocyanin and ferredoxin-NADP reductase are added to the chloroplasts. Similarly, the steady state rate of the

[$\text{H}_2\text{O} \longrightarrow \text{DPIP}$] Hill reaction (B) is increased about threefold by added MgCl_2 and plastocyanin, but added ferredoxin or ferredoxin-NADP reductase have no effect on this reaction. Plastocyanin appears to be the electron transport component which couples to DPIP, either in the oxidized or in the reduced form, in the reaction media. The steady state rate of the [$\text{H}_2\text{O} \longrightarrow \text{NADP}$] reaction (C) with saturating amounts of ferredoxin can be further increased more than threefold when MgCl_2 , plastocyanin and ferredoxin-NADP reductase are added.

INTRODUCTION

In the evolution of models based on the original proposal by Hill and Bendall (1) of the two-light reaction or Z scheme for higher plant photosynthesis, two contrasting pictures of the organization and function of the chlorophyll pigments have emerged (2,3). In the first of these, the "separate package" model, each light reaction is thought to be associated with its own array of light-absorbing pigment molecules, and no communication at the level of electronic excitation transfer is permitted between the two arrays. It is as if the two light reactions, each with associated pigment system, are physically separated from one another. In the second, or "spillover" model, the pigment arrays are still distinct, but electronic excitation transfer is permitted between them in order to make up for deficiencies in the activation of one or the other light reactions. The series two-light scheme of Hill and Bendall requires that both light reactions be driven at equal rates for optimum overall photosynthetic efficiency. An extreme modification of the "spillover" hypothesis, in which a major portion of the chlorophyll pigments is common to both photosystems, has been proposed by Avron and Ben-Hayyim (4). In this common pool one finds all, or nearly all, of the bulk pigments absorbing in the red region; i.e., at wavelengths shorter than about 680 nm. A common feature of all of the models is the assignment of far-red ($\lambda > 700$ nm) absorption almost exclusively to photosystem 1. This is thought to be responsible for the "red-drop" in the quantum yield of photosynthesis at long wavelengths first observed by Emerson and Lewis (5,6).

There are several ways in which these models can be tested experimentally and in which they predict different results. One approach is to make careful measurements of quantum yields at different wavelengths for each light reaction operating in the absence of each other. According to the "separate package" hypothesis, photons absorbed by the pigment system of the functioning light reaction can be used photochemically and those photons absorbed by the pigments of the non-operative photosystem will necessarily be wasted. The latter will result in fluorescence or heat, but never in any photochemistry. By contrast, in the "spillover" hypothesis, photons absorbed in either pigment system have, by virtue of the possibility of electronic excitation transfer, the potential ability to do photochemistry. As pointed out in a previous publication (7), the "separate package" hypothesis requires that at a given wavelength of activation the sum of the quantum yields for the two light reactions measured separately ($\phi_I + \phi_{II}$) can never exceed 1.0 equivalents-(einstein absorbed)⁻¹. According to the "spillover" hypothesis, the sum of quantum yields may exceed 1.0, and may approach a limiting value of 2.0 in the case of perfectly efficient electronic excitation transfer.

Until recently, such studies of quantum yield spectra have not produced convincing evidence of sums exceeding 1.0 (7-9). Unfortunately, this result is not conclusive, as it is always possible to argue that the chloroplasts under investigation were partially inactivated during preparation and this resulted in artificially low yields. Now Avron and Ben-Hayyim (4) have presented experimental findings in which the quantum yield at 640 nm for the ferricyanide Hill reaction,

probably associated solely with photosystem 2, is 1.0 and that for DCMU-blocked photoreduction of NADP by ascorbate/DPIP₂, a photosystem 1 reaction, is about 0.5. With diquat or FMN as the electron acceptor even higher values were reported. Because these results appeared to be in conflict with findings previously reported from this laboratory, we have undertaken a new study of this question. Using different assay systems and making extensive efforts to optimize chloroplast activities, we have now obtained results which are in essential agreement with those of Avron and Ben-Hayyim (4). A clear consequence of these findings is that the simple "separate package" hypothesis is no longer tenable. Which of several versions of the "spill-over" hypothesis is correct is a much more difficult question to answer.

An alternative approach to this question can be made by investigations of the Emerson enhancement effect on isolated chloroplasts. Results of such studies, which generally confirm the conclusions referred to above, are the subject of the following paper.

MATERIALS AND METHODS

Spinach and preparation of chloroplasts

Chloroplasts were isolated from six to eight week old spinach plants grown from seed in a growth chamber (10); Spinacia oleracea var. early hybrid No. 7 was used. In order to obtain the maximum photochemical capability, the spinach leaves were picked two to six hours after the start of the illumination cycle in the growth chamber and just before the isolation procedure. The leaves were rinsed with

cold distilled water, ribs were removed, and the leaves were then stored at -20°C for 10 min. Ten g of leaves was homogenized between 10 and 15 sec in 50 ml of 0.5 M sucrose, 0.1 M tricine, pH 7.6 buffer in a Waring blender. The resultant homogenate was strained through 8 layers of cheesecloth and centrifuged at 200 g for 1 min. The supernatant was then centrifuged at 1000 g for 10 min and the precipitate resuspended in 1 ml of 0.5 M sucrose, 0.05 M tricine pH 7.6 and stored at 0°C . All the above procedures were carried out at 0°C . Optimal activity of the chloroplasts could be obtained only within the first 6 hours following isolation.

Reagents

NADP was obtained from P-L Biochemical, Inc., Milwaukee, Wisconsin; DPIP and tricine from Sigma Chemical Co., St. Louis, Missouri; sodium ascorbate from Calbiochem, Los Angeles, California; sucrose from J. T. Baker Chemical Co., New Jersey; DCMU from E. I. DuPont de Nemours, Wilmington, Delaware; nitrogen gas directly from liquid nitrogen, obtained from Pacific Oxygen Co., Oakland, California..

Apparatus and light intensity measurements

Quantum yield measurements at various activation wavelengths were carried out using a modified Cary Model 14 spectrophotometer, as described by Sauer and Biggins (9) for photoreduction of NADP, and by Sauer and Park (10) for photoreduction of DPIP. Measurements of light intensity were based on secondary standard lamps in the same way as Sauer and Biggins (9). The actinic light was obtained from a Bausch & Lomb monochromator (red-blazed grating) with supplementary

cut-off filters (10). Monochromator slit widths were set at 3 mm, resulting in an actinic band width of 10 nm. The quantity of light absorbed by chloroplasts was measured using the same method as Sauer and Biggins (9).

Preparation of ferredoxin, NADP reductase and plastocyanin

Ferredoxin was prepared from commercial spinach according to the procedures of Tagawa and Arnon (11). The ratio of absorbances at 420 nm and 274 nm of purified ferredoxin was 0.445. Ferredoxin-NADP reductase and plastocyanin were isolated in the same preparation as was ferredoxin. The NADP reductase was fractionated from the DEAE cellulose column at a concentration of $(\text{Cl}^-) = 0.12 \text{ mole-}\ell^{-1}$ and plastocyanin at $(\text{Cl}^-) = 0.26 \text{ mole-}\ell^{-1}$. Both NADP reductase and plastocyanin were used without further purification with ammonium sulfate.

Reaction mixtures

The reaction mixture for the $[\text{ascorbate} + \text{DPIP} \xrightarrow{\text{H}_2\text{O}} \text{NADP}]$ reaction contained the following in $\mu\text{mole-ml}^{-1}$: tricine (pH = 7.5), 45; MgCl_2 , 7.5; DPIP, 0.053; DCMU, 0.01; sodium ascorbate, 5.0; NADP, 0.67; ferredoxin, $33.3 \mu\text{g-ml}^{-1}$; NADP reductase, saturating amount or not added; plastocyanin, saturating amount, or not added; chlorophyll, $13 \mu\text{g-ml}^{-1}$.

The reaction mixture for the $[\text{H}_2\text{O} \xrightarrow{\text{DPIP}} \text{NADP}]$ reaction contained the following in $\mu\text{mole-ml}^{-1}$: tricine (pH = 7.5), 45; MgCl_2 , 4.5; DPIP, 0.053; plastocyanin, saturating amount; chlorophyll, $13 \mu\text{g-ml}^{-1}$.

The reaction mixture for the $[\text{H}_2\text{O} \xrightarrow{\text{NADP}} \text{NADP}]$ reaction contained the following in $\mu\text{mole-ml}^{-1}$: tricine (pH = 7.5), 45; MgCl_2 , 7.5;

ferredoxin, $67 \mu\text{g}\cdot\text{ml}^{-1}$; NADP, 0.67; NADP reductase, saturating amount; plastocyanin, saturating amount; chlorophyll, $13.5 \mu\text{g}\cdot\text{ml}^{-1}$.

Procedures for obtaining anaerobic conditions

Three ml of reaction mixture was prepared in a small test tube (0.5 x 6 cm). The chloroplasts were added to the reaction mixture in the dark. The test tube was covered with a tight rubber cap and shaken well. The reaction mixture was flushed with nitrogen while shaking for 2 min. The nitrogen was passed through syringe-needles penetrating the rubber cap on the test tube. The reaction mixture was transferred quickly with a nitrogen-flushed syringe into two cuvettes, on which rubber caps had been tied firmly with string, and then flushed with nitrogen through syringe-needles for 2 min. These procedures were carried out in the dark.

Measurement of chlorophyll concentrations

The measurement of total chlorophyll and chlorophyll a and b concentrations (12,13) are according to the following equations, where the chlorophylls are in 80% acetone solutions:

$$\text{Chl } (\underline{a+b}) (\mu\text{g}\cdot\text{ml}^{-1}) = 2.9 \times 10^{-2} \times (A_{652 \text{ nm}}) \quad (1)$$

$$\text{Chl } \underline{a} (\mu\text{g}\cdot\text{ml}^{-1}) = 11.63 \times (A_{665 \text{ nm}}) - 2.39 (A_{649 \text{ nm}}) \quad (2)$$

$$\text{Chl } \underline{b} (\mu\text{g}\cdot\text{ml}^{-1}) = 20.11 (A_{649 \text{ nm}}) - 5.18 (A_{665 \text{ nm}}) \quad (3)$$

where A_λ = the absorbance of the chlorophyll solution in 80% acetone at wavelength λ .

RESULTS

(1) Photosystem I: [Ascorbate + DPIPH₂ → NADP] reaction with no added plastocyanin or NADP reductase

Typical time course for the photoreduction of NADP. The typical time course for the photoreduction of NADP at high light intensity by isolated chloroplasts for the [ascorbate + DPIPH₂ → NADP] reaction is shown in Fig. 1. It shows that there is a rapid initial burst just after turning on the actinic light, as had been reported previously (9). This suggests that there is a reservoir of electrons that can be rapidly transferred to NADP via photosystem I, and that the rate-limiting step is then the replenishment of this reservoir by the ascorbate/DPIPH₂ couple. The second portion of the slope is initially linear, corresponding to the steady-state photoreduction of NADP in this system. There is a rapid small reversal at the time of turning off the exciting light, which may result from the reverse oxidation of NADPH in the dark by the depleted reservoir of reducing power.

Primary quantum requirement. The intensity dependence of the quantum requirement for the photoreduction of NADP is shown in Fig. 2. The intrinsic quantum requirements are obtained by extrapolation to zero incident intensity. In most cases the quantum requirements obtained from the steady-state rate of the photoreduction of NADP at different intensities lie fairly well on a straight line. The quantum requirements obtained from the rapid initial rates are plotted separately in Fig. 2. They also lie in a straight line, but with a smaller slope. The primary quantum requirements at zero

intensity, obtained from either initial rates or steady-state rates, are identical. The rapid initial phase disappears at the lowest intensities, where light absorption becomes the rate-limiting step.

Action spectrum. The spectrum of the zero-intensity quantum requirements for the photoreduction of NADP in the [ascorbate + DPIPH₂ → NADP] reaction is given as the solid curve in Fig. 3. The quantum requirements at wavelengths above 700 nm are near 1.0. Removal of oxygen does not produce any change in the quantum requirements or in the rate of NADP photoreduction in this system.

Effect of ferredoxin. Ferredoxin is a protein containing non-heme iron and has one of the most negative redox potentials in chloroplasts (14). It is known to be readily water soluble and essential to the electron transport chain. The rate of photoreduction of NADP was less than $\Delta A_{340 \text{ nm}} (\text{min})^{-1} = 0.002$ when no ferredoxin was added to the reaction mixture together with the isolated chloroplasts. If other factors, such as light intensity, DPIP and NADP concentrations, etc., were not rate-limiting, the rate of photoreduction of NADP increased to $\Delta A_{340 \text{ nm}} (\text{min})^{-1} = 0.062$ as a linear function of the concentration of added ferredoxin, which increased from zero to 13 $\mu\text{g/ml}$ in the reaction mixture. The rate of NADP photoreduction reached a saturation at about $\Delta A_{340 \text{ nm}} (\text{min})^{-1} = 0.084$ when the ferredoxin concentration was 27 $\mu\text{g}\cdot\text{ml}^{-1}$ of reaction mixture. In our standard reaction mixture 100 μg of ferredoxin in 3 ml of reaction mixture was used.

Effect of oxygen. It was thought that a portion of the electrons transported by photosystem I in the [ascorbate + DPIPH₂ → NADP]

reaction would leak to oxygen via ferredoxin (14). For this reason, the reaction was studied under anaerobic conditions. Experiments showed, on the other hand, that the presence of air had no effect on the rates or quantum requirements of this reaction. Apparently the leak of electrons to oxygen in this system is negligible under our experimental conditions.

(2) Photosystem I: [Ascorbate + DPIPH₂ → NADP] reaction using saturating amounts of plastocyanin and NADP reductase

Plastocyanin. Plastocyanin is a copper-containing protein in chloroplasts and is readily water soluble. The addition of plastocyanin to the spinach chloroplast preparation restores part of what was lost during the isolation process. If other factors such as light intensity, ferredoxin concentration, etc., are not rate-limiting, steady state the rate of photoreduction of NADP⁺ in the [ascorbate + DPIPH₂ → NADP] system can be increased more than two fold when optimal amounts of plastocyanin are added, and this incident intensity is 3.0 nanoeinstein-cm⁻²-sec⁻¹ at 678 nm (or absorbed intensity is 0.23 nanoeinstein-(μg chl)⁻¹-sec⁻¹). The relationship between the rate of photoreduction of NADP and plastocyanin concentration for this system is shown in Fig. 4.

Ferredoxin-NADP reductase. Ferredoxin-NADP reductase is another readily water soluble protein in chloroplasts and is essential to the photosystem I electron transport chain leading to NADP (14). With optimal concentrations of ferredoxin and plastocyanin, the rate of photoreduction of NADP can be increased further by added ferredoxin-

NADP reductase. The relationship between the rate and the NADP reductase concentration is shown in Fig. 5. When the absorbed intensity was $0.23 \text{ nanoeinstein-(}\mu\text{g chl)}^{-1}\text{-sec}^{-1}$, or incident intensity was $3.0 \text{ nanoeinstein-(cm)}^{-2}\text{-sec}^{-1}$ at 678 nm, the steady state rate of NADP photoreduction, ΔA at 340 nm $(\text{min})^{-1}$, was 0.058 when no plastocyanin or NADP reductase was added, and increased to 0.115 when optimal plastocyanin was added, and to 0.21 when both optimal plastocyanin and NADP reductase were present. The final rate with optimal plastocyanin and NADP reductase is more than three times greater than the rate without these additions.

Primary quantum requirements and action spectrum. Fig. 6 shows the intensity dependence of the $[\text{ascorbate} + \text{DPIP} \xrightarrow{\text{h}\nu} \text{NADP}]$ reaction of chloroplasts in the presence of saturating amounts of both plastocyanin and ferredoxin-NADP reductase. Comparison with Fig. 2, where reaction conditions were similar except that no plastocyanin or reductase were added, shows that the reaction proceeds about 2.5 times faster in the former case at high intensities, but that both sets of data extrapolate to nearly the same quantum requirements at zero intensity, from 2.3 to 2.5 (einsteins absorbed)-equivalent $^{-1}$.

Saturating amounts of plastocyanin and ferredoxin-NADP reductase had no significant effect on the zero intensity quantum requirements for the $[\text{ascorbate} + \text{DPIP} \xrightarrow{\text{h}\nu} \text{NADP}]$ reaction for activation wavelengths between 620 and 720 nm. The general agreement is shown in Fig. 3.

(3) Photosystem II: $[\text{H}_2\text{O} \xrightarrow{\text{h}\nu} \text{DPIP}]$ reaction

pH Dependence. In order to obtain the optimum rate, the photoreduction of DPIP was studied from pH 6.0 to 9.0, using 45 mM of tricine

as the buffer to adjust the pH. The dependence of the photoreduction rate of DPIP on pH is shown in Fig. 7. Five to seven independent experiments were carried out at each pH. Although the extinction coefficient of DPIP decreases below pH 7 (ref. 15), we compare only the measured rate of change of absorbance at 580 nm. As shown in Fig. 7, the optimum rate of photoreduction of DPIP is at pH 7.5.

DPIP concentration. Since DPIP has an absorption maximum at 600 nm with a broad spectrum in the visible region, its presence diminishes the effective absorption of chloroplasts. Therefore we tried to find the optimum DPIP concentration, which will not screen the absorption of chloroplasts too much but will approach saturation as a substrate. The concentration-dependence of the rate of photoreduction of DPIP is shown in Fig. 8. A rather sharp maximum in the photoreduction rate occurs at a DPIP concentration of 2.7×10^{-5} mole- ℓ^{-1} , and the activity falls off rather quickly at higher concentrations. In a previous study, where brief flashes of illumination were used, the activity remained high to 6×10^{-5} mole- ℓ^{-1} of DPIP (16). This indicates that the pattern and intensity of illumination are important factors in determining the optimal concentrations of substrates.

Magnesium chloride. Both magnesium and chloride ions are essential for photosynthesis. As in other reactions involving the transfer of a phosphate group, Mg^{++} is required for phosphorylation by chloroplasts (17). We found that by adding magnesium chloride the rate of the $[H_2O \longrightarrow DPIP]$ reaction could be increased about sixty percent over the rate in the absence of added magnesium chloride (Fig. 9).

Plastocyanin. Since plastocyanin has been implicated in non-cyclic electron transport from water to NADP (18-20) and also is readily water soluble, we investigated its effect on the rate of photoreduction of DPIP in the $[H_2O \rightarrow DPIP]$ reaction at incident intensity about $3.5 \text{ nanoeinstein-cm}^{-2}\text{-sec}^{-1}$ at 678 nm. Fig. 10 shows that the rate of photoreduction of DPIP is increased more than forty percent when saturated plastocyanin is added. Similar increases were observed in six separate experiments. After optimizing all the above conditions, the steady state rate of photoreduction of DPIP was increased about threefold from $\Delta A \text{ at } 580 \text{ nm (min)}^{-1} = 0.28$ to 0.70. The incident intensity was near $3.0 \text{ nanoeinstein-cm}^{-2}\text{-sec}^{-1}$ throughout this part of the investigation.

Ferredoxin and NADP reductase. Both ferredoxin and NADP reductase were investigated as to their effects on the photoreduction of DPIP by the chloroplasts. Each of these components was added, separately or together, at different concentrations to the reaction mixture. No significant improvement on the photoreduction rate of DPIP was found when low concentrations of either (or both) ferredoxin or NADP reductase were added. A small decrease in the rate occurred when higher concentrations of either or both of them were added. Apparently, they are not involved in this part of the electron transport chain.

Action spectrum of the quantum requirement. When the rate of photoreduction of DPIP was optimized, the quantum requirements of this reaction were measured over a range of light intensities at several wavelengths. The action spectrum of the zero-intensity quantum requirements is shown in Fig. 11. The values at each

wavelength are averaged from the five to seven independent experiments where chloroplasts were most active, from a total of more than fifteen experiments performed. Quantum requirements of 1.0 ± 0.05 were obtained when the actinic light was between 630 and 660 nm. Higher values of the quantum requirement were obtained when the actinic light was at 670 nm or longer wavelengths.

(4) Photosystem (I and II): $[H_2O \rightarrow NADP]$ reaction

Optimum rate of photoreduction of NADP. The rate of photoreduction of NADP by chloroplasts, as for the other reactions, is dependent on pH, the concentration of magnesium chloride, plastocyanin, ferredoxin, and NADP reductase, method of preparation of chloroplasts and the age of spinach. Experiments have been carried out similar to those for the $[H_2O \rightarrow DPIP]$ reaction in order to optimize the reaction rate of the system. The optimum conditions are given as the standard reaction mixture in the Materials and Methods section. Magnesium chloride, ferredoxin, plastocyanin and NADP reductase are all essential for this reaction. High rates of photoreduction of NADP were obtained from pH 7.0 to 8.0, with a maximum at pH 7.5, which was used in our experiment. The rate of the reaction when saturating ferredoxin was added was twenty-fold more than the rate when no ferredoxin was added. With the optimum concentration of $MgCl_2$, $7.5 \mu\text{moles-ml}^{-1}$, the rate could be increased about thirty percent from ΔA at $340 \text{ nm} \cdot (\text{min})^{-1} = 0.065$ to 0.090 , over the rate when no $MgCl_2$ was added.

With the optimum concentration of $MgCl_2$, ferredoxin and pH the steady state photoreduction rate of NADP of the reaction was more than doubled

by adding saturating NADP reductase, and more than tripled by adding both saturating NADP reductase and plastocyanin. These experiments were carried out using an incident light intensity about $2.9 \text{ nanoeinstein-cm}^{-2}\text{-sec}^{-1}$ at 678 nm.

Action spectrum of quantum requirements. The action spectrum of zero-intensity quantum requirements for the $[\text{H}_2\text{O} \rightarrow \text{NADP}]$ reaction is shown in Fig. 12. Only the optimized conditions for the reaction have been used for obtaining the quantum requirements. The results shown in Fig. 12 are the values obtained from the best five to seven independent experiments where chloroplasts were most active from a total of more than fifteen experiments performed. When chloroplasts were not active we were not able to obtain these low quantum requirements. The quantum requirements from 620 to 660 nm and at 678 nm of actinic light are very close to 2.0 ± 0.1 , and they are slightly higher at 670 and 690 nm. The photoreduction of NADP becomes very inefficient when the actinic light is at 700 nm or longer wavelengths.

DISCUSSION

The normal course of photosynthetic electron transport in higher plant chloroplasts leads to the reduction of NADP to NADPH and the oxidation of water to molecular oxygen. A current version of the mechanism of this process, based on the series two-light reaction scheme originally proposed by Hill and Bendall (1) and elaborated for the $[\text{H}_2\text{O} \rightarrow \text{NADP}]$ reaction by Losada, et al. (21), is shown in Fig. 13. DCMU is a potent inhibitor of the reduction of NADP (22), and this

inhibition can be relieved, in part, by the addition of the couple $\text{DPIP} \cdot \text{H}_2$ plus ascorbate (23). Based on the action spectrum of quantum requirements, Hoch and Martin (8) and Sauer and Biggins (9) suggested that the photoreduction of NADP^+ in the $[\text{ascorbate} + \text{DPIP} \cdot \text{H}_2 \rightarrow \text{NADP}]$ reaction is associated solely with light reaction I. In the absence of DCMU, the oxidized form of DPIP serves as an electron acceptor for the chloroplast Hill reaction, leading to the photo-oxidation of water to molecular oxygen. This reaction has been assigned to light reaction II alone, on the basis of action spectra and the absence of enhancement in the presence of long wavelengths of light, but it has not been possible to rule out the possibility that light reaction I participates as well. A detailed discussion of this question was given by Sauer and Park (10). On the basis of the results of the present study, where quantum yields of 1.0 equivalent- $(\text{einstein absorbed})^{-1}$ are observed for the $[\text{H}_2\text{O} \rightarrow \text{DPIP}]$ reaction using actinic wavelengths from 630 to 660 nm, we can now unambiguously assign this reaction to light reaction II alone. If both light reactions were involved, then at least two absorbed photons would be required for each electron transferred and the quantum yield would never exceed 0.5 at any actinic wavelength. From the analogous results of Avron and Ben-Hayyim (4), the ferricyanide Hill reaction can also be assigned to light reaction II alone. In our studies of the DPIP Hill reaction, the absence of any effect resulting from the addition of ferredoxin and/or ferredoxin-NADP reductase supports the assignment to light reaction II.

The effect of added plastocyanin in increasing the rate of electron transport in the $[H_2O \rightarrow \text{DPIP}]$ reaction at moderate light intensities suggests that the DPIP accepts electrons close to photosystem I in broken chloroplasts. Presumably added plastocyanin is relieving a rate limiting step in this reaction under conditions where light is not limiting. This observation is consistent with the finding that methylamine, an uncoupler of non-cyclic photophosphorylation, also serves to accelerate electron flow in the $[H_2O \rightarrow \text{DPIP}]$ reaction (10,16). The site of non-cyclic photophosphorylation is thought to occur between photosystem II and plastocyanin. We believe that the influence of added plastocyanin on this system II reaction indicates that DPIP accepts electrons either directly from plastocyanin or else from an adjacent electron transport component whose oxidation state is controlled by plastocyanin.

Associated with each of the two photosynthetic reaction centers is an array of light absorbing or antenna pigment molecules (chlorophylls a and b and carotenoids in the case of higher plants). Many models have been proposed to describe the arrangement of these molecules (2-4). In one model, the "separate package" hypothesis, each pigment molecule is associated with one or the other of the light reactions and no communication, by way of electronic excitation transfer, is permitted between them. As a consequence of this model, photons absorbed by a particular pigment molecule can be transferred only to the reaction center of its own pigment system. If that reaction center happens not to be functioning, then the excitation will be wasted and will appear only as fluorescence or heat. If we

measure the quantum yields at low light intensities, where the arrival of photons is rate limiting, for each of the light reactions operating separately, then according to the "separate package" hypothesis the sum of the quantum yields at each wavelength should not exceed 1.0 (ref. 7). In Table I we present the zero intensity quantum yields at several wavelengths between 620 and 720 nm for the [ascorbate + DPIP $\longrightarrow$ NADP] reaction attributed to photosystem I and for the [$\text{H}_2\text{O} \longrightarrow$ DPIP] reaction attributed to photosystem II. The sums of the two quantum yields at each wavelength are given in column 4. Although the sums are reasonably close to unity at long wavelengths (670 to 710 nm) they exceed unity by 45 to 60% in the shorter wavelength range (630 to 660 nm) of activating light. Light reaction II itself has a quantum yield near unity in this wavelength range, and the quantum yield of light reaction I is around 0.5. These results, together with those reported previously by Avron and Ben-Hayyim (4), are clearly inconsistent with the "separate package" hypothesis and suggest that it is no longer tenable.

An alternative model, the "spillover" hypothesis, includes the possibility that excitation present in one of the pigment systems may, if it is not needed there, be transferred to the other reaction center and used there. The efficiency of transfer may depend on the wavelength of activation, on the conditions of the reaction and on the rates of competing excitation-loss processes. In an extreme version of the "spillover" hypothesis, proposed by Avron and Ben-Hayyim (4), the distinction between the two pigment systems disappears (at least at all but the longest wavelengths) and photons absorbed

anywhere in the antenna may ultimately reach either type of reaction center. It should be recognized that proximity of a given antenna pigment molecule to a reaction center may be important in determining the probability of transfer of its excitation, particularly where the time required for all of the necessary transfer steps to occur is comparable with the chlorophyll fluorescence lifetime. These spillover models, in common with most others, assume that the longest wavelengths of activating light involve photons with insufficient energy to activate the reaction center of light reaction II.

If there is a single pigment system absorbing in the red region (630 to 660 nm) and if both types of reaction center can receive excitation from this pigment system, then we might expect the quantum yields for both light reaction I and for light reaction II to be the same for these actinic wavelengths. Our measurements show, however, that the quantum yield of light reaction II is about twice as large as that for light reaction I in the red region. This suggests that the two types of reaction centers are not symmetrically disposed with respect to the antenna pigments. When reaction center I is not operating because of the absence of suitable electron acceptors (NADP and/or ferredoxin) all excitation in the red region is able to be trapped and utilized for light reaction II photochemistry. On the other hand, when light reaction II is blocked by DCMU, only half the red photons absorbed are capable of producing electron transfer in light reaction I. We know that this is not due to inactivation of system I reaction centers, because they are fully effective for long wavelength photons (700 to 720 nm). Also, in order to account for a

quantum yield of 0.5 at short wavelengths for the $[H_2O \rightarrow NADP]$ reaction, where both light reactions operate in series, it is necessary to assume that the photons absorbed are partitioned equally between the two light reactions and that each light reaction is able to use essentially all of the excitation that it receives.

One measure of the fate of excitation not utilized by light reaction I is the fluorescence associated with photosystem II. This fluorescence, which has an emission spectrum that is consistent with its origin in photosystem II pigments, has been interpreted as containing a "live" portion and a "dead" portion (24). The "live" portion is seen to compete with photochemistry carried out by light reaction II and attains a maximum efficiency when photochemistry is saturated at high light intensities. The "dead" portion, on the other hand, contributes a constant yield of fluorescence that is independent of the state of photosystem II. If this indeed is the case, then it is unlikely that the "dead" fluorescence comes from the same site as is responsible for the "live" fluorescence and for photochemistry. The "dead" fluorescence may come from a non-functional trap or from the antenna pigment molecules themselves. In either case, it represents a constant leak of excitation energy from the chloroplast membranes. The addition of DCMU, which blocks photosystem II light reactions, causes the "live" fluorescence to rise to its maximum value. This suggests that DCMU does not close the traps of light reaction II as energy sinks. In fact they appear to operate just as efficiently in this respect in the presence or in the absence of DCMU. This observation is difficult to reconcile with our quantum

yield measurements, for virtually all of the short wavelength photons are trapped by light reaction II in the $[H_2O \rightarrow \text{DPIP}]$ reaction in the absence of DCMU, whereas in the presence of DCMU in the $[\text{ascorbate} + \text{DPIP} \rightarrow \text{NADP}]$ reaction at least half of these short wavelength photons must reach light reaction I. Yet the maximum yield of "live" fluorescence from photosystem II is the same in both cases.

Perhaps the simplest way around this apparent inconsistency is in the alternative model of Avron and Ben-Hayyim (4) in which a control mechanism determines the fraction of excitation that reaches each reaction center. This control mechanism is under the influence of factors which differ from one set of reaction conditions to another. The most likely candidates for this factor are the redox potential of the system and the concentrations of added cofactors. Experiments by Murata (25) suggest that Mg^{++} concentration plays a role in excitation transfer among the antenna pigments; however; the effects observed here could result from a complex interaction among several such components. Additional results reported by Avron and Ben-Hayyim (4), where flavin mononucleotide (FMN) or diquat are the electron acceptors for the photosystem I reaction, show that in these cases 640 nm photons are fully effective in producing electron transfer. This contrasts sharply with the $[\text{ascorbate} + \text{DPIP} \rightarrow \text{NADP}]$ reaction and is further evidence that some control mechanism is determining the fraction of red light excitation that is available to photosystem I.

In summary, the results of quantum yield measurements for the separate photosystem I and photosystem II reactions show that a simple separate package model for the antenna pigment molecules is inconsistent

with the experimental findings. The variability of the fraction of photons in the red region of the spectrum that is available to each of the reaction centers suggests the presence of a control mechanism that regulates electronic excitation transfer among major portions of the antenna pigment molecules. The investigation and identification of the mechanism of this "controlled spillover" hypothesis will be an important field for future research.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Dr. Charles Weiss, Jr. for stimulating discussions concerning the relationship of these results to the models of the photosynthetic membranes. The investigations described in this paper were sponsored, in part, by the U. S. Atomic Energy Commission.

REFERENCES

- (1) R. Hill and F. Bendall, *Nature* 186, 136 (1960).
- (2) J. Myers and J. Graham, *Plant Physiol.* 38, 105 (1963).
- (3) L. N. M. Duysens and J. Amesz, *Biochim. Biophys. Acta* 64, 243 (1962).
- (4) M. Avron and B. Ben-Hayyim, Progress in Photosynthesis Research, vol. III, H. Metzner, Ed., Univ. of Tübingen Press, Germany, 1969, pg. 1185.
- (5) R. Emerson and C. M. Lewis, *J. Gen. Physiol.* 25, 579 (1942).
- (6) R. Emerson and C. M. Lewis, *Am. J. Bot.* 30, 165 (1943).
- (7) J. Kelly and K. Sauer, *Biochemistry* 4, 2798 (1965).
- (8) G. Hoch and I. Martin, *Arch. Biochem. Biophys.* 102, 430 (1963).
- (9) K. Sauer and J. Biggins, *Biochim. Biophys. Acta* 102, 55 (1965).
- (10) K. Sauer and R. B. Park, *Biochemistry* 4, 2791 (1965).
- (11) K. Tagawa and D. I. Arnon, *Nature* 195, 537 (1962).
- (12) G. Mackinney, *J. Biol. Chem.* 140, 315 (1941).
- (13) H. H. Strain and W. A. Svec in The Chlorophylls, L. P. Vernon and G. R. Seely, Eds., Academic Press, New York, 1966, pg. 51.
- (14) D. I. Arnon, *Science* 149, 1460 (1965).
- (15) J. McD. Armstrong, *Biochim. Biophys. Acta* 86, 194 (1964).
- (16) J. Kelly and K. Sauer, *Biochemistry* 7, 882 (1968).
- (17) D. I. Arnon, *Brookhaven Symposia in Biology* 11, 181 (1958).
- (18) S. Kato and A. Takamiya, *Biochim. Biophys. Acta* 99, 156 (1965).
- (19) S. Kato and A. San Pietro, in The Biochemistry of Copper, J. Peisach et al., Eds., Academic Press, New York, 1966, pg. 407.
- (20) D. S. Gorman and R. P. Levine, *Plant Physiol.* 41, 1648 (1966).

- (21) M. Losada, F. R. Whatley and D. I. Arnon, *Nature* 190, 806 (1961).
- (22) N. I. Bishop, *Biochim. Biophys. Acta* 27, 205 (1958).
- (23) L. P. Vernon and W. S. Zaugg, *J. Biol. Chem.* 235, 2728 (1960).
- (24) R. K. Clayton, *Biophys. J.* 9, 60 (1969).
- (25) N. Murata, *Biochim. Biophys. Acta* 189, 171 (1969).

TABLE I

Quantum Yields Extrapolated to Zero Intensity for NADP Reduction by [Ascorbate + DPIP] and by Water and for the DPIP Hill Reaction of Spinach Chloroplasts at Various Wavelengths.

Wavelength (nm)	Quantum yields (equivalents/einstein absorbed)			
	[Ascorbate + DPIP $\rightarrow$ NADP] ϕ_I	[H ₂ O $\rightarrow$ DPIP] ϕ_{II}	$\phi_I + \phi_{II}$	[H ₂ O $\rightarrow$ NADP] $\phi_{(I+II)}$
620	0.67 $\pm$ 0.04			0.50 $\pm$ 0.02
630	0.61 $\pm$ 0.04	1.00 $\pm$ 0.04	1.61 $\pm$ 0.08	0.50 $\pm$ 0.02
640	0.53 $\pm$ 0.04	0.95 $\pm$ 0.05	1.48 $\pm$ 0.09	0.49 $\pm$ 0.02
650	0.51 $\pm$ 0.03	1.00 $\pm$ 0.05	1.51 $\pm$ 0.08	0.50 $\pm$ 0.02
660	0.50 $\pm$ 0.03	0.96 $\pm$ 0.05	1.46 $\pm$ 0.08	0.48 $\pm$ 0.03
670	0.43 $\pm$ 0.03	0.63 $\pm$ 0.05	1.06 $\pm$ 0.08	0.45 $\pm$ 0.05
678	0.38 $\pm$ 0.11	0.56 $\pm$ 0.06	0.94 $\pm$ 0.17	0.50 $\pm$ 0.03
690	0.61 $\pm$ 0.04	0.44 $\pm$ 0.03	1.05 $\pm$ 0.07	0.43 $\pm$ 0.04
700	1.00 $\pm$ 0.05	0.25 $\pm$ 0.03	1.25 $\pm$ 0.08	0.19 $\pm$ 0.03
710	0.96 $\pm$ 0.05	0.18 $\pm$ 0.03	1.14 $\pm$ 0.08	0.16 $\pm$ 0.01
720	0.96 $\pm$ 0.05			

FIGURE CAPTIONS

Fig. 1. Typical time course for the photoreduction of NADP by isolated chloroplasts in the [ascorbate + DPIP_H₂ → NADP] reaction. The reaction mixtures are given in the text, but plastocyanin and NADP reductase were not added. Actinic light at 678 nm; incident intensity 3.0 nanoeinsteins-cm⁻²sec⁻¹; chlorophyll concentration 13 µg·ml⁻¹. The molar absorptivity for NADPH at 340 nm is 6.2 x 10³ l·mole⁻¹·cm⁻¹.

Fig. 2. The relationship between the quantum requirement for NADP reduction and incident intensity in the [ascorbate + DPIP_H₂ → NADP] reaction; no plastocyanin or NADP reductase added. Circles: obtained from the steady-state rate; triangles: obtained from the rapid initial phase. Illumination and chlorophyll concentration as in Fig. 1.

Fig. 3. Action spectrum of the zero-intensity quantum requirement of photoreduction of NADP in the [ascorbate + DPIP_H₂ → NADP] reaction, with and without added plastocyanin (PCy) and NADP reductase (Fp). Upper curve: sample without added plastocyanin or NADP reductase, shown as —I—. Lower curve: sample with added saturating plastocyanin and NADP reductase, shown as -- $\frac{1}{2}$ --. Saturating ferredoxin was added to both reaction mixtures; other components are given in the text.

Fig. 4. The influence of plastocyanin concentration on the rate of photoreduction of NADP⁺ in the [ascorbate + DPIP_H₂ → NADP] reaction. The reaction mixture is given in the text; plastocyanin concentration unknown. Actinic light at 678 nm; incident intensity 2.8 nanoeinstein-cm⁻²·sec⁻²; chlorophyll concentration 13 µg·ml⁻¹.

Fig. 5. The influence of plastocyanin and NADP reductase concentrations on the rate of photoreduction of NADP^+ in the $[\text{ascorbate} + \text{DPIP} \xrightarrow{\text{h}\nu} \text{NADP}]$ reaction. Upper curve: with added plastocyanin in saturating amounts, $\circ$. Lower curve: without added plastocyanin, Δ . Absolute concentrations of plastocyanin and reductase are unknown. Illumination conditions and chloroplast concentration as in Fig. 4.

Fig. 6. The relationship between the quantum requirement of NADP^+ photoreduction and incident intensities in the $[\text{ascorbate} + \text{DPIP} \xrightarrow{\text{h}\nu} \text{NADP}]$ reaction with saturating amounts of plastocyanin (PCy) and NADP reductase (Fp). Illumination conditions and chloroplast concentration as in Fig. 4.

Fig. 7. The pH dependence of the rate of photoreduction of DPIP in the reaction $[\text{H}_2\text{O} \xrightarrow{\text{h}\nu} \text{DPIP}]$. Results obtained from five to seven independent experiments at each pH. The reaction mixture is given in the text, except that no plastocyanin was added in these experiments. Illumination conditions and chloroplast concentration as in Fig. 4.

Fig. 8. The rate of photoreduction of DPIP as a function of DPIP concentration. The reaction mixture is given in the text, except that no plastocyanin was added. Illumination conditions and chloroplast concentration as in Fig. 4.

Fig. 9. The rate of photoreduction of DPIP as a function of MgCl_2 concentration. The reaction mixture is given in the text, except that no plastocyanin was added. Illumination conditions and chloroplast concentration as in Fig. 4.

Fig. 10. The rate of photoreduction of DPIP as a function of plastocyanin concentration. The reaction mixture is given in the text. Plastocyanin was isolated from spinach; its absolute concentration was unknown. Illumination conditions and chloroplast concentration as in Fig. 4.

Fig. 11. Spectrum of the quantum requirements of photoreduction of DPIP in the $[H_2O \rightarrow DPIP]$ reaction, extrapolated to zero light intensity.

Fig. 12. Spectrum of the quantum requirements of photoreduction of $NADP^+$ in the $[H_2O \rightarrow NADP]$ reaction, extrapolated to zero light intensity.

Fig. 13. Electron transport scheme for the two light reaction mechanism in chloroplasts.

XBL 7012-5490

XBL 7012-5500

Figure 3

XBL 7012-5497

XBL 7012-4900

XDL 7012-5491

Figure 6

XBL 7012-5496

XBL 7012-5499

Figure 9

XBL 7012-5498

Figure 11

XBL 7012-5492

XBL 7012-5495

XBL 7012-5493

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

- A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or*
- B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.*

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

TECHNICAL INFORMATION DIVISION
LAWRENCE RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720