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Photosystem Electron-Transport Capacity and Light-Harvesting Antenna Size in Maize Chloroplasts[1]

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ABSTRACT

Spectrophotometric and kinetic measurements were applied to yield photosystem (PS) stoichiometries and the functional antenna size of PSI, $PSII_\alpha$, and $PSII_\beta$ in *Zea mays* chloroplasts *in situ*. Concentrations of PSII and PSI reaction centers were determined from the amplitude of the light-induced absorbance change at 320 and 700 nm, which reflect the photoreduction of the primary electron acceptor Q of PSII and the photooxidation of the reaction center P700 of PSI, respectively. Determination of the functional chlorophyll antenna size (N) for each photosystem was obtained from the measurement of the rate of light absorption by the respective reaction center. Under the experimental conditions employed, the rate of light absorption by each reaction center was directly proportional to the number of light-harvesting chlorophyll molecules associated with the respective photosystem. We determined $N_{P700} = 195$, $N_\alpha = 230$, $N_\beta = 50$ for the number of chlorophyll molecules in the light-harvesting antenna of PSI, $PSII_\alpha$, and $PSII_\beta$, respectively. The above values were used to estimate the PSII/PSI electron-transport capacity ratio (C) in maize chloroplasts. In mesophyll chloroplasts $C > 1.4$, indicating that, under green actinic excitation when Chl *a* and Chl *b* molecules absorb nearly equal amounts of excitation, PSII has a capacity to turn over electrons faster than PSI. In bundle sheath chloroplasts $C < 1$, suggesting that such chloroplasts are not optimally poised for linear electron transport and reductant generation.

Under light-limiting conditions, the measured rate of PSI and PSII electron transport in chloroplasts is a function of the rate of excitation transfer to the reaction center and the number of reaction centers in the thylakoid membrane. The rate of excitation transfer to the reaction center of a photosystem (K) depends on three parameters: the intensity of the actinic excitation (I), the effective absorption cross-section of the light-harvesting pigments of that PS[2] (σ), and the number of antenna Chl molecules transferring excitation to the photochemical reaction center (N). On the basis of these considerations, it is evident that photosynthetic systems may have three possible mechanisms to modulate rates of electron transport *in vivo*: (a) by changing the relative concentrations of PSI and PSII complexes, (b) by changing the physical antenna size of the PS, or (c) by varying the Chl composition of the antenna of a reaction center which may affect the absorption cross-section parameter σ. For the reasons stated above, it is important to develop experimental procedures by which to test PS stoichiometry, physical antenna size, and antenna composition of chloroplast samples.

Over the past three years, evidence has accumulated suggesting that higher plant chloroplasts do not have a fixed stoichiometry of PSI and PSII reaction centers. This notion was supported both by spectrophotometric (16, 18, 28) and by O_2 flash yield measurements (7, 23, 24). Of particular interest is the observation that environmental light conditions and mutations may have a significant effect both in the chloroplast ultrastructure and in the ratio of PSII/PSI reaction centers (18, 22).

The determination of the physical antenna size of chloroplast PS *in situ* is more difficult to establish. Ley and Mauzerall (12) have employed an O_2 flash yield method by which they determined the absorption cross-section of PSII in *Chlorella vulgaris*. A method developed in this laboratory, based on the steady-state kinetic measurements of Q photoreduction and P700 photooxidation, has permitted the estimation of the antenna size of both PSI and PSII *in situ* (15, 22, 28).

In the present work we applied spectrophotometric and kinetic measurements to obtain PS stoichiometries and the functional antenna size of PSI, $PSII_\alpha$, and $PSII_\beta$ in maize chloroplasts. We also present a quantitative evaluation of the overall electron transport capacity (C) of maize chloroplast PS, and compare it to the values reported for C_3 plants.

MATERIALS AND METHODS

Leaves from 2- to 3-week-old *Zea mays L.* plants, grown under controlled conditions in the greenhouse, were cut in small pieces and ground for 40 s in a Waring Blendor at 0°C, in 50 mM Tricine buffer, pH 7.9, which contained 0.4 M sucrose, 10 mM NaCl, and 5 mM $MgCl_2$. The slurry was filtered through 8 layers of cheesecloth; cell debris were removed by centrifugation at 1,000*g* for 3 min. Chloroplasts were precipitated at 6,000*g* for 7 min and were resuspended in a small volume of the same buffer. Chl concentration and Chl *a*/Chl *b* ratios were determined in 80% acetone (3).

The concentration of the primary electron acceptor Q of PSII was determined from the amplitude of the light-minus-dark absorbance change at 320 nm with DCMU-treated chloroplasts in the presence of 2.5 mM potassium ferricyanide. We used the procedure and equations of Pulles *et al.* (26) to correct for particle flattening effects and applied a differential extinction coefficient of 13 mM^{-1} cm^{-1} to the corrected absorbance difference ΔA_{320} amplitude (29). The flattening correction factor was in the range of 1.2 to 1.3 for our maize chloroplast samples. The concentration of the reaction center P700 of PSI was determined from the amplitude of the light-minus-dark absorbance change at 700 nm of solubilized chloroplast membranes suspended in the presence of 2 mM sodium ascorbate and 200 μM methyl viologen. An extinction coefficient of 64 mM^{-1} cm^{-1} was used for the calculation of P700 concentration (10).

The rate of light absorption by PSII was determined under light-limiting conditions from the analysis of the area growth

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[2] Abbreviations: PS, photosystem; Q, primary electron acceptor of PSII; P700, reaction center of PSI; LHC, light-harvesting complex.

over the fluorescence induction curve of DCMU and hydroxylamine-treated chloroplasts (19). The rate of light absorption by PSI was determined from the analysis of the kinetics of the absorbance change at 700 nm, under the same excitation conditions (15). To eliminate secondary electron transport to $P700^+$ from plastocyanin, Cyt *f* and plastoquinone, the chloroplasts were pretreated with KCN which blocks plastocyanin function (14, 25). Chloroplasts were preincubated for 20 min in the presence of 25 mM KCN in a medium containing 20 mM Tricine, pH 7.8, 10 mM NaCl, 5 mM $MgCl_2$, and 200 mM sucrose. Following the KCN pretreatment, the chloroplasts were washed and resuspended in the isolation buffer. Signal averaging was performed with a Tracor Northern NS-570A. The kinetic analysis of the fluorescence induction was performed by an on-line Hewlett Packard HP85A computer interfaced with a high speed voltmeter.

Actinic excitation of uniform field was provided by broad band green light transmitted by a combination of CS 4-96 and CS 3-70 Corning filters (10% transmittance at 503 nm and 590 nm, maximum of 60% transmittance at 533 nm) at an intensity of 120 $\mu E\ m^{-2}\ s^{-1}$. Green actinic illumination was used in order to provide more nearly equal excitation of Chl *a* and Chl *b* molecules (see below).

Theoretical. To derive a rate equation for the electron transport process through a given photosystem, we would first consider an isolated photochemical reaction center illuminated with incident light of intensity I in the wavelength range of λ_1 to λ_2. The excitation and subsequent electron transfer process at the reaction center (RC) is represented by the following reaction mechanism:

$$RC \xrightarrow{\sigma \cdot I} RC^* \rightarrow RC^+ + e^- \quad (1)$$

where

σ = optical cross section of the reaction center,
I = actinic light intensity.

The second step represents the electron transfer reaction and is extremely fast, on the order of picoseconds, so the overall rate of the electron transfer reaction is limited by the rate of the first step which, under continuous illumination, represents the rate of light absorption by the RC. Thus, the measured rate of electron transfer in a sample containing reaction centers would depend on the rate of light absorption. The rate equation for the electron transport process at the reaction center is given by the following equation:

$$\frac{d(e^-)}{dt} = K' \cdot [RC] = \sigma \cdot \phi \cdot I \cdot [RC] \quad (2)$$

where

K' = the apparent rate constant of electron transfer by RC
σ = absorption cross-section of RC in the wavelength range λ_1 to λ_2
ϕ = fraction of excited molecules that undergo photochemistry (quantum yield of photochemistry)
I = intensity of the actinic excitation.

A chloroplast photosystem is composed of a reaction center surrounded by a number *N* of light-harvesting Chl molecules arranged such as to absorb the light energy and transfer the excitation efficiently to the reaction center. Then, the rate of electron transport by the RC of that photosystem will be *N* times faster than that derived for the isolated RC (equation 2) and will be given by:

$$\frac{d(e^-)}{dt} = K \cdot [RC] = N \cdot \sigma \cdot \phi \cdot I \cdot [RC] \quad (3)$$

where

K = the apparent rate constant of electron transfer by the PS
N = number of light-harvesting Chl molecules.

In practice, there are two kinds of light-harvesting Chl molecules, Chl *a* and Chl *b*. Since the number of Chl *a* and Chl *b* molecules is variable for PSI and PSII and also because their optical cross-sections show dissimilar wavelength dependence, we would need to expand equation 3 to account for such features. The equation for the apparent rate constant of electron transport by a reaction center would then be modified to:

$$K = (N_{Chla} \cdot \sigma_{Chla} + N_{Chlb} \cdot \sigma_{Chlb}) \cdot \phi \cdot I \quad (4)$$

where

N_{Chla} = number of light-harvesting Chl *a* molecules
N_{Chlb} = number of light-harvesting Chl *b* molecules
$N = N_{Chla} + N_{Chlb}$.

It is clear, however, that if a photosystem is excited with light that is absorbed equally by Chl *a* and Chl *b*, then $\sigma_{Chla} = \sigma_{Chlb}$ and the rate constant *K* is simplified to that shown in equation 3, in which case the photosystem can be treated as homogeneous with respect to the light-harvesting population of *N* Chl molecules.

In order to determine antenna size of the photosystems in the thylakoid membrane, we need measure the apparent rate constant of electron transport (*K*) by a given photosystem and then use the following system of equations in the determination of the number *N* of Chl molecules transferring excitation energy to each photosystem:

$$K_{P700} = N_{P700} \cdot \sigma \cdot \phi \cdot I \quad (5)$$

$$K_\alpha = N_\alpha \cdot \sigma \cdot \phi \cdot I \quad (6)$$

$$K_\beta = N_\beta \cdot \sigma \cdot \phi \cdot I \quad (7)$$

If all other parameters are the same in the three equations, then a direct comparison of rate constants *K* will be a direct comparison of antenna size *N*. The actinic intensity *I* would be the same in the three cases as long as chloroplast samples are excited with the same light intensity when recording rate measurements. The quantum yield (ϕ) of photochemistry at each PS has been reported to be greater than 0.8 (4, 6, 12, 27, 28). For the purpose of this work we assumed equal quantum yields for $PSII_\alpha$, $PSII_\beta$, and PSI. Given the fact that each PS has different Chl composition, the parameter σ will be kept the same in the three cases only under actinic excitation that is absorbed equally by Chl *a* and Chl *b*. As mentioned previously, if $\sigma_{Chla} = \sigma_{Chlb}$ then the light-harvesting antenna of the PS can be treated as a homogeneous population of *N* molecules such that $N_{Chla} + N_{Chlb} = N$.

Provided that the above constraints apply, equations 5 to 7 will be simplified to:

$$K_{PSI} = c \cdot N_{PSI} \quad (8)$$

$$K_{PSII_\alpha} = c \cdot N_{PSII_\alpha} \quad (9)$$

$$K_{PSII_\beta} = c \cdot N_{PSII_\beta} \quad (10)$$

where $c = \sigma \cdot \phi \cdot I$ and is the same in the three equations.

In determining the apparent rate constants of electron transport by the chloroplast PS we used green actinic light (transmitted by a combination of CS 4-96 and CS 3-70 Corning filters) to provide as equal as possible excitation of individual Chl *a* and Chl *b* molecules. This contention was tested by measuring the effective absorption of light by a Chl *a* and a Chl *b* molecule in the wavelength range transmitted by the actinic filter. Under our experimental conditions, the effective absorption of light by a Chl molecule would be the product of the extinction coefficient (specific absorbance) of the pigment times the transmittance of the actinic filter (effective absorption = Chl extinction coefficient × filter transmittance).

Determination of the effective absorption spectrum of a Chl *a* and a Chl *b* molecule *in vivo* is difficult to obtain because of the uncertainty in Chl specific absorbance values. We approached this problem by using the specific absorbance values of purified Chl *a* and Chl *b* in ether (30). Figure 1 shows the effective absorption spectra of Chl *a* and Chl *b* when excited with green light. It is clear that a Chl *b* molecule would absorb more green light than a Chl *a* molecule in ether. Table I compares the integrated absorption of light by Chl *a* and Chl *b* under broad band green actinic illumination. For ether-solubilized pigments, a Chl *b* molecule would absorb 28% more green light than a Chl *a* molecule. The specific absorbance bands of Chl *a* and Chl *b* in ether are sharper than those *in vivo* and, in addition, they are shifted to shorter wavelengths (9). To illustrate the significance of the Chl microenvironment in the above derivations, we compared the Chl *a* and Chl *b* integrated absorption in acetone (Fig. 1). We used the specific absorbance spectra given by Mackinney (13) for acetone-solubilized pigments. The results are also presented in Table I and show that Chl *b* in acetone would absorb about 18% more green light than Chl *a*. The analysis presented in Table I clearly shows that the overall absorption of green light by Chl *a* and Chl *b* depends on the pigment microenvironment.

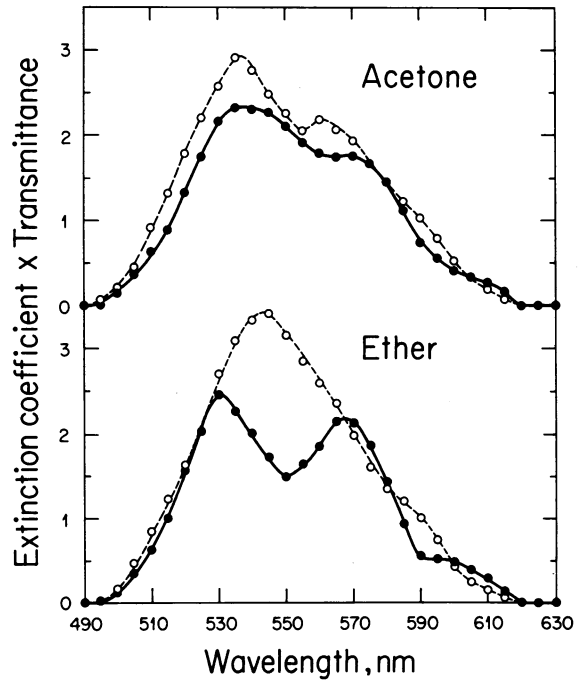

FIG. 1. Effective absorption spectra of ether and acetone solubilized Chl *a* (●) and Chl *b* (○) excited with green actinic light (CS 4-96 and CS 3-70 Corning filters). The effective absorption of a Chl molecule at each wavelength is given by the product of the extinction coefficient (specific absorbance) of the pigment $\epsilon(\lambda)$ and the transmittance $T(\lambda)$ of the actinic light filter. We used specific absorbance values for ether-solubilized Chl molecules from Zscheile and Comar (30); specific absorbance values for acetone-solubilized Chl molecules were derived from Mackinney (13).

Table I. *Effect of Pigment Microenvironment on the Integrated Effective Absorption of Light by a Chl a and a Chl b Molecule*

Integrated effective absorption values ($\int_{\lambda_1}^{\lambda_2} \epsilon[\lambda]\cdot T[\lambda]\cdot d\lambda$) were calculated from the area below the effective absorption spectra for Chl *a* and Chl *b* molecules in ether and acetone (Fig. 1), respectively. Green (CS 4-96 and CS 3-70 Corning filters) actinic excitation was used. The last column shows the percent difference in integrated effective absorption of light between Chl *b* and Chl *a* (Δ[Chl *b*-Chl *a*]).

Pigment Solvent	$\int_{\lambda_1}^{\lambda_2} \epsilon(\lambda)\cdot T(\lambda)\cdot d\lambda$		Δ(Chl *b*-Chl *a*)
	Chl *a*	Chl *b*	
			%
Ether	2.01	2.56	28
Acetone	2.01	2.37	18

The purpose of the above considerations was to arrive at an estimate of the imbalance in green light absorption between PSI and PSII due to dissimilar Chl composition: it is known that about 36% of the antenna Chl of PSII is Chl *b* (11, 15). The corresponding number is much smaller (about 14% of Chl *b*) for the light-harvesting antenna Chl of PSI (2, 15). Therefore, the difference in Chl *b* content between PSII and PSI is approximately 22%. Assuming temporarily that under our experimental conditions Chl *b* would absorb 28% more green light than Chl *a*, as it would in ether solution, we estimated that PSII would receive at most 6% (0.28 × 0.22) more excitation energy than if PSII and PSI contained equal amounts of Chl *b* in their light-harvesting pigment. By the same reasoning, for Chl molecules in acetone, PSII would receive at most 4% (0.18 × 0.22) more excitation than if PSII and PSI contained equal numbers of Chl *b* in their antenna.

The specific absorbance values of Chl *a* and Chl *b* *in vivo* are not known. *In vivo*, Chl molecules are embedded in a hydrophobic protein matrix which is integral to the thylakoid membrane. Due to multiple associations of Chl molecules with protein, the absorbance spectra of photosynthetic membranes lack the sharp absorbance bands of ether or acetone-solubilized pigments. Indeed, absorbance spectra of chloroplasts show multiple bands due to different states of Chl *a* and Chl *b* interaction with protein molecules (5). Under *in vivo* conditions, therefore, it is likely that integrated absorption of light by a Chl *a* and a Chl *b* molecule may not be as different as those presented in Table I.

Thus, the excitation imbalance between PSI and PSII, arising because of their dissimilar Chl composition, could be less than the value of 4% to 6% quoted above. On the basis of the above considerations, under continuous green illumination, the rate of light absorption by each PS would be directly proportional to the number of associated Chl molecules, independent of the Chl composition.

RESULTS

The isolation method used in this work yielded maize chloroplast samples with a Chl *a*/Chl *b* ratio ranging from 3.5 to 4.0, indicating a mixture of mesophyll and bundle sheath chloroplasts (8). We have shown in a previous work (8) that the functional photosynthetic unit size of PSI in maize mesophyll and bundle sheath chloroplasts is the same. This permitted us to treat a sample containing both types of chloroplasts as homogeneous with respect to PSI. Mesophyll chloroplasts contained mostly $PSII_\alpha$, with $PSII_\beta$ comprising a small (30%) fraction of the total PSII. On the other hand, bundle sheath chloroplasts contained $PSII_\beta$ only (8). Fluorescence induction kinetics of chloroplast samples containing either only mesophyll chloroplasts or a mixture of both mesophyll and bundle sheath chloroplasts were biphasic, reflecting the photoactivity of $PSII_\alpha$ and $PSII_\beta$ (17, 19). The amplitude of the two kinetic components of PSII depended on the relative enrichment in bundle sheath and mesophyll chloroplasts. However, the rate constants of the two kinetic components were independent of the chloroplast composition of our samples. Thus, with samples enriched in bundle sheath chloroplasts which contained mainly $PSII_\beta$ we measured the same photoactivity rate constant K_β as in mesophyll chloroplasts. This was taken as evidence that the antenna size of $PSII_\beta$ in both mesophyll and bundle sheath chloroplasts is the same, and that a mixed sample containing both mesophyll and bundle sheath chloroplasts is homogeneous with respect to $PSII_\beta$ as well.

The rate of light absorption by a reaction center is directly proportional to the number N of Chl molecules transferring excitation energy to that reaction center, *i.e.* $K = c \cdot N$, where c is a proportionality constant depending on the yield of photochemistry at each reaction center (ϕ), on the effective absorption cross section (σ) of the antenna Chl and on the intensity (I) of the actinic excitation. On the basis of the considerations presented in the theoretical section, the equation $K = c \cdot N$ can be applied to both PSI and PSII with the same c value. Under identical green illumination conditions, the rate constant of electron transfer K would be a measure of the rate of light absorption by a PS, and it would vary linearly as a function of the number N of Chl molecules transferring excitation energy to that PS. As such, K would be a direct measure of N. In this work we measured the rate of light absorption K by PSI and PSII directly and have calculated the number N of Chl molecules transferring excitation to each PS.

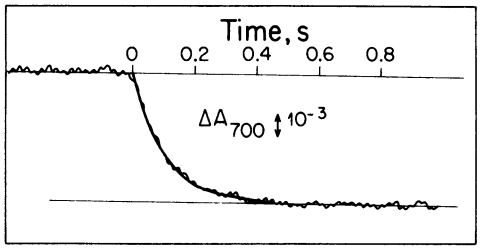

FIG. 2. Kinetics of P700 photooxidation induced by weak continuous green actinic light (120 μE m^{-2} s^{-1}) which came ON at zero time. The reaction mixture contained 310 μM Chl from a chloroplast sample with Chl a/Chl b = 4; 40 μM DCMU and 250 μM methyl viologen. The optical pathlength of the cuvette for the measuring beam was 0.21 cm.

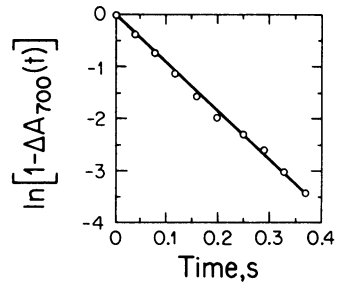

FIG. 3. Semilogarithmic analysis of the absorbance change kinetics shown in Figure 2. The rate of light absorption by P700 (K_{P700}) is given from the slope of the straight line, K_{P700} = 9.3 s^{-1}.

In our maize chloroplast samples we measured total Chl (a + b) per Q ratio (Chl/Q) of about 320 and the Chl/P700 ratio of about 460, yielding a Q/P700 (PSII/PSI) ratio of 1.4. The above stoichiometric values reflect a mixture of PSII-enriched mesophyll and PSI-enriched bundle sheath chloroplasts. Figure 2 shows the kinetics of P700 photooxidation induced by green actinic excitation. Under light-limiting conditions, the rate of P700 photooxidation is limited by the rate of light absorption by PSI. Figure 3 shows a semilogarithmic plot of the P700 photooxidation kinetic curve, which is a monophasic function of time occurring with a rate (K_{P700}) of approximately 9.3 s^{-1}. Figure 4 shows the fluorescence induction curve of maize chloroplasts in the presence of DCMU. The variable part of the fluorescence induction provides a measure of the rate of system II photoactivity (reduction of Q). It consists of two kinetic components of which the fast component is attributed to the photoactivity of $PSII_\alpha$ and $PSII_\beta$ and the slow β-component reflects the photoactivity of $PSII_\beta$ only (17, 19–21). The photoreduction kinetics of Q were also monitored directly at 320 nm, using DCMU-treated thylakoids in the presence of potassium ferricyanide. Figure 5 shows the photoreduction of Q as a function of time. This is also a biphasic process, which will be deconvoluted into two independent kinetic components (see below).

Figure 6A (●) shows a semilogarithmic plot of the area over the fluorescence induction curve as a function of time. The slow phase in Figure 6A is linear with a rate constant (K_β) of 2.4 s^{-1}. The intercept of this straight line with the ordinate at zero time provided an estimate of the contribution of $PSII_\beta$ in the measurement. We estimated this contribution to be approximately 26% of the total, which translated into relative values of $PSII_\alpha$/PSI = 1.06 and $PSII_\beta$/PSI = 0.36. A kinetic expression of $PSII_\alpha$ activity was determined by subtracting the contribution of the slow β-component from the total area growth kinetics. Figure 6B (●) shows a semilogarithmic plot of the photoactivity kinetics of $PSII_\alpha$. As reported earlier (19, 21), the fast α-component is nonlinear with time, and a rate of light absorption by $PSII_\alpha$ (K_α) is obtained from the initial slope (at zero time) of the curve in Figure 6B. Under our experimental conditions, the rate constant K_α was approximately 10.7 s^{-1} for maize chloroplast samples.

Figure 6A (○) shows a semilogarithmic plot of the kinetics of ΔA_{320}. It clearly shows a slow exponential β-component ($PSII_\beta$) with rate of 2.3 s^{-1} and a faster component which is nonexponential. The β-component in the ΔA_{320} kinetics contributed to approximately 29% of the total absorbance change in our chloroplast preparation, as shown by the y-intercept of the slow linear phase. Figure 6B (○) shows the semilogarithmic plot of the kinetics of the fast α-component. The initial slope (at zero time) was used to calculate K_α, the rate of light absorption by $PSII_\alpha$

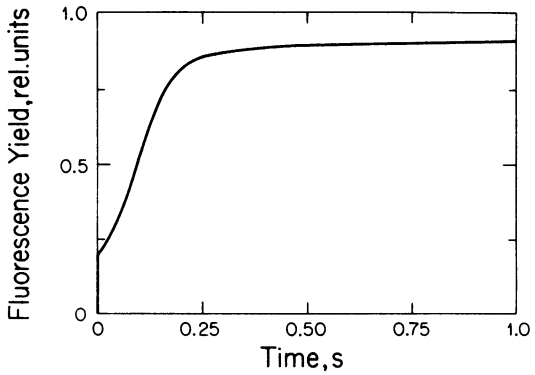

FIG. 4. Fluorescence induction kinetics of maize chloroplasts under green actinic illumination. The reaction mixture contained 107 μM Chl from a chloroplast sample with Chl a/Chl b = 3.4; 40 μM DCMU and 1 mM hydroxylamine. The maximal fluorescence yield F_{max} = 0.88.

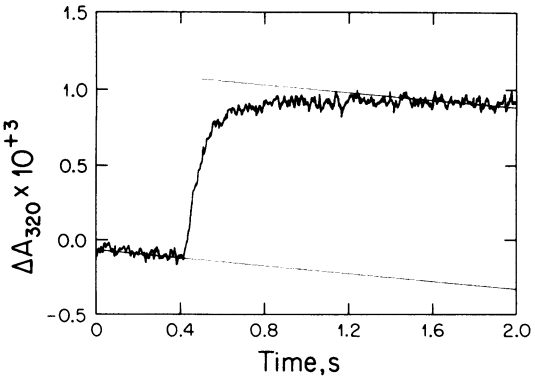

FIG. 5. Kinetics of Q photoreduction induced by weak continuous green actinic light (120 μE m^{-2} s^{-1}) which came ON at 0.4 s. The reaction mixture contained 250 μM Chl from a chloroplast sample with Chl a/Chl b = 3.6; 40 μM DCMU and 2.5 mM potassium ferricyanide.

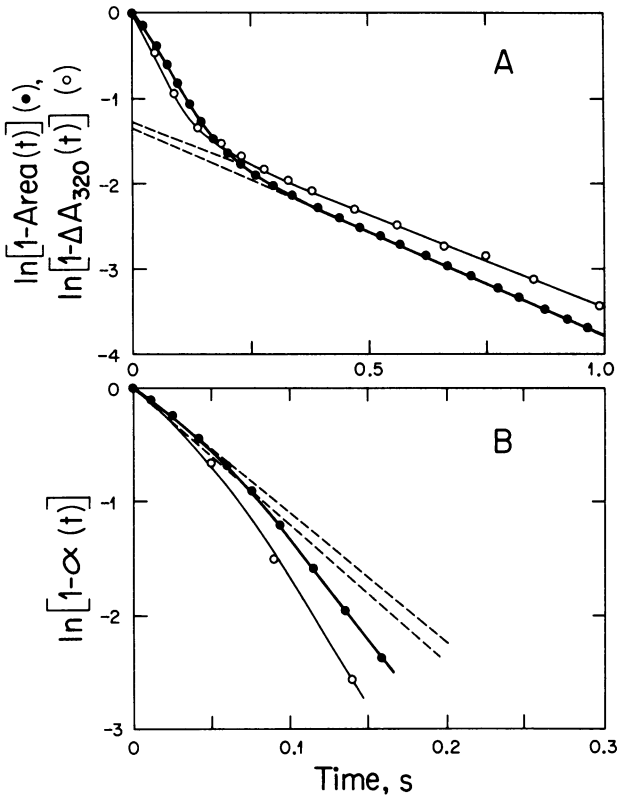

FIG. 6. A, Semilogarithmic analysis of the growth of the area over the fluorescence induction curve (●) from Figure 4, and of the absorbance change kinetics (○) shown in Figure 5. Note the biphasic shape of both plots, containing a fast phase reflecting the activity of $PSII_\alpha$ and $PSII_\beta$ and a slower linear phase reflecting the activity of $PSII_\beta$ only. The slope of the linear phase (- - -) defined K_β, the rate of light absorption by $PSII_\beta$, while its intercept with the ordinate at zero time provided a direct measure of the relative concentration of $PSII_\beta$ in the reaction mixture. $PSII_\beta$ contributed 26% and 29% to the total area growth and total absorbance change, respectively. $K_\beta = 2.3\ s^{-1}$ as determined from the fluorescence kinetic analysis and $K_\beta = 2.4\ s^{-1}$ from Q photoreduction kinetic analysis. B, Semilogarithmic analysis of the fast kinetic component ($PSII_\alpha$) of the area over the fluorescence induction curve (●) and of the ΔA_{320} absorbance change kinetics (○), calculated by subtacting the contribution of the slow β-component from the total area growth and from the total absorbance change kinetics, respectively. The rate of light absorption by $PSII_\alpha$ (K_α) was obtained from the initial slope of each curve (slope at zero time, - - -). $K_\alpha = 11\ s^{-1}$ as determined from the fluorescence kinetic analysis and $K_\alpha = 12\ s^{-1}$ as determined from Q photoreduction kinetic analysis.

($K_\alpha = 12\ s^{-1}$).

The similarity between the photoactivity kinetic parameters of PSII measured indirectly from the area over fluorescence induction and directly from the ΔA_{320} kinetics suggested similar Chl *a* fluorescence yields from the pigment beds of $PSII_\alpha$ and $PSII_\beta$. Fluorescence yield measurements, therefore, can be effectively used to monitor PSII activity in higher plant chloroplasts (17, 21).

Table II summarizes average values of the rates of light absorption for PSI (K_{P700}), $PSII_\alpha$ (K_α) and $PSII_\beta$ (K_β) obtained from a number of measurements such as described above. As stated earlier, the relationship between rate of light absorption (K) and functional photosynthetic unit size (N) of a PS is given by equations 8 to 10.

The total Chl to P700 ratio (Chl/P700 = 460) of our chloroplast samples is not a meaningful measure of the photosynthetic unit size, but it represents a statistical average of all Chl ($a + b$) molecules, some of which are associated exclusively with PSII. The Chl/P700 ratio is a function of the number N of antenna Chl molecules of each photosystem, which is given below:

$$\frac{\text{Chl}}{\text{P700}} = \frac{[PSII_\alpha]\cdot N_\alpha}{[PSI]} + \frac{[PSII_\beta]\cdot N_\beta}{[PSI]} + N_{P700} \qquad (11)$$

From the solution of the system of equations 8 to 11, we calculated the values of N_{P700}, N_α, and N_β in maize chloroplasts:

$$N_{P700} = 195$$

$$N_\alpha = 230$$

$$N_\beta = 50.$$

Table II. *Rate of Light Absorption by PSI (K_{P700}), $PSII_\alpha$ (K_α) and $PSII_\beta$ (K_β)*

Rates are determined from the kinetics of P700 photooxidation, Q photoreduction, and fluorescence induction analysis induced by weak green actinic illumination. The relative concentration of $PSII_\beta$ is given as per cent of the total PSII present in the sample.

K_{P700}	K_α	K_β	$PSII_\beta$
s^{-1}	s^{-1}	s^{-1}	%
9.3 ± 0.7	11.2 ± 1.0	2.4 ± 0.4	26 ± 11

DISCUSSION

Linear electron transport from H_2O to $NADP^+$ requires the cooperation of a number of electron transfer chains in which nonstoichiometric amounts of PSII and PSI reaction centers are connected (16). The rate of electron transport through each reaction center depends on their respective antenna size and is directly proportional to the incident light intensity. Under saturating light, the electron transport rate is limited by the oxidation of the plastoquinone pool, a reaction with rate constant on the order of 100 s^{-1}. However, under our light-limiting conditions (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) the rates of light absorption by PSI and PSII are one order of magnitude smaller, *i.e.* around 10 s^{-1}, suggesting that the oxidation rate of plastoquinone will not be the rate-limiting step in photosynthetic electron transport.

In a chloroplast sample, the electron turnover by all PS complexes in the thylakoid membrane is determined from the concentration of that PS and from the rate of light absorption by its reaction centers. We obtained an estimate of the overall rate of electron flow through PSII and PSI in our chloroplast samples from the products of the rate of light absorption K by a PS times the concentration [PS] of that photosystem; $R = K \cdot [PS]$. This basic equation would allow the estimation of the rate of electron turnover under any experimental condition, and it can be used to estimate the electron transport capacity ratio between the two photosystems:

$$C = \frac{R(PSII_\alpha) + R(PSII_\beta)}{R(PSI)} = \frac{K_\alpha[PSII_\alpha] + K_\beta[PSII_\beta]}{K_{P700}[PSI]} \qquad (12)$$

In "Results" we demonstrated the accurate determination of the value of the rate of light absorption and of the concentration of each PS. Equation 12, therefore, could be used under a variety of experimental conditions to determine whether overall electron flow through PSII is greater, equal, or less than that through PSI. The usefulness of equation 12 is that it will help in determining conditions for a balanced electron flow through the photosynthetic electron transport chain.

Under our green excitation conditions and assuming [PSI] = 1, solution of equation 12 yielded the following ratio:

$$C = \frac{11.2 \times 1.06 + 2.4 \times 0.26}{9.3} = 1.34$$

suggesting that capacity for electron turnover by PSII is greater than that of PSI by 1.34:1 in our chloroplast sample. As discussed in "Results", our chloroplast sample was a mixture of mesophyll and bundle sheath chloroplasts. Since bundle sheath chloroplasts lack $PSII_\alpha$ and are enriched in PSI (8), we would expect the electron transport capacity ratio C of pure bundle sheath chloroplasts to be much lower than 1.0, reflecting the absence of $PSII_\alpha$. On the other hand, $PSII_\alpha$-containing mesophyll chloroplasts would exhibit an electron transport capacity ratio C higher than 1.34. In agreement, spinach chloroplasts showed a PS electron-transport capacity ratio C (PSII/PSI) of about 1.8 (15). Thus, it appears that C_4 plants like maize, which have probably evolved from C_3 plants, have retained the basic photochemical apparatus organization and function of the C_3 plants. This is evidenced both in the relative photosystem stoichiometric ratios and in the relative photosystem antenna size.

The relative concentrations of $PSII_\alpha$, $PSII_\beta$, and PSI in grana-containing mesophyll chloroplasts and agranal bundle sheath chloroplasts from maize correlate well with the different functions performed by the two types of chloroplasts in a C_4 plant. Grana-containing mesophyll chloroplasts perform linear electron transport like the C_3 chloroplasts and contain the full complement of the various electron transport components. Agranal bundle sheath chloroplasts show some PSII activity (1) and are responsible for CO_2 fixation; they lack $PSII_\alpha$ and its associated Chl a/b LHC and most of the Cyt b_{559} but were shown to contain $PSII_\beta$ and the full complement of PSI and Cyt f (8). The photosynthetic unit size of each photosystem, however, is the same in mesophyll and bundle sheath chloroplasts, suggesting that genetic information coding for PSI and PSII is retained both in mesophyll and bundle sheath chloroplasts. One may conclude, therefore, that the differentiation of bundle sheath chloroplasts during the development of the maize leaf involves an as yet unknown environmental or biochemical signal responsible for selectively switching off the genome coding for the structural and functional components of grana.

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