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STRUCTURAL AND FUNCTIONAL ORGANIZATION OF THE PHOTOSYSTEMS IN SPINACH CHLOROPLASTS

ANTENNA SIZE, RELATIVE ELECTRON-TRANSPORT CAPACITY, AND CHLOROPHYLL COMPOSITION

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The structural and functional organization of the spinach chloroplast photosystems (PS) I, II_α and II_β was investigated. Sensitive absorbance difference spectrophotometry in the ultraviolet (ΔA_{320}) and red (ΔA_{700}) regions of the spectrum provided information on the relative concentration of PS II and PS I reaction centers. The kinetic analysis of PS II and PS I photochemistry under continuous weak excitation provided information on the number (N) of chlorophyll (Chl) molecules transferring excitation energy to PS II_α, PS II_β and PS I. Spinach chloroplasts contained almost twice as many PS II reaction centers compared to PS I reaction centers. The number N_α of chlorophyll (Chl) molecules associated with PS II_α was 234, while $N_\beta = 100$ and $N_{\mathrm{PS\,I}} = 210$. Thus, the functional photosynthetic unit size of PS II reaction centers was different from that of PS I reaction centers. The relative electron-transport capacity of PS II was significantly greater than that of PS I. Hence, under light-limiting green excitation when both Chl *a* and Chl *b* molecules are excited equally, the limiting factor in the overall electron-transfer reaction was the turnover of PS I. The Chl composition of PS I, PS II_α and PS II_β was analyzed on the basis of a core Chl *a* reaction center complex component and a Chl *a*/*b*-LHC component. There is a dissimilar Chl *a*/*b*-LHC composition in the three photosystems with 77% of total Chl *b* associated with PS II_α only. The results indicate that PS II_α, located in the membrane of the grana partition region, is poised to receive excitation from a wider spectral window than PS II_β and PS I.

Introduction

One of the outstanding contributions in photosynthetic research was the demonstration by Emerson and Arnold [1,2] that Chl *a* and other accessory pigments acted cooperatively to collect and trap light energy. The concept of the photosynthetic unit proposed by Gaffron and Wohl [3] explained the results of Emerson and Arnold by proposing the structural and functional organization of light-harvesting pigments in the form of units around the photochemical reaction center. Later, the photosynthetic unit was defined either as the total number of chlorophyll molecules per P-700 [4,5] or cytochrome *f* [6], since it was assumed that there was a fixed stoichiometry of

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, photosystem; P-700, reaction center of PS I; Q, primary electron acceptor of PS II; LHC, light-harvesting complex; Tricine, *N*-tris(hydroxymethyl)methylglycine.

P-680:cytochrome *f*:P-700 of 1 : 1 : 1. The concept of the photosynthetic unit has been used widely because of its significance in relation to photosynthetic electron transport. The photosynthetic unit size is intimately related to the electron-transport capacity of the chloroplast. Under light-limiting conditions, the electron-transport capacity of chloroplasts depends strictly on the capacity of each photosystem for electron turnover. In turn, the overall electron-transport capacity of each photosystem depends on the relative concentration of the particular photosystem in the chloroplast, on the light-harvesting antenna size, i.e., the number of Chl *a* and Chl *b* molecules that transfer excitation energy to the photosystem, and on the chlorophyll composition of its light-harvesting antenna.

The recognition of the varying stoichiometries of PS II and PS I reaction centers and of the varying antenna size of PS II and PS I [7–10] required a reassessment of the definition of the photosynthetic unit [9–11]. It was proposed that the photosynthetic unit size of PS II may be different from that of PS I and each must be determined separately [9]. Furthermore, there are two functionally and structurally independent types of PS II, PS II_α and PS II_β. The antenna unit of PS II_α is larger than that of PS II_β and several PS II_α units are organized in a connected package, while PS II_β units are separate from each other [12,13]. The hypothesis that the two types of PS II units were located in separate membrane regions [12,14] has recently been substantiated [15], since PS II_α was located in the membrane of the grana partitions only, whereas PS II_β was exclusively located in stroma-exposed thylakoids [15].

Recent results with higher plant chloroplasts suggest that in addition to varying stoichiometries of PS II and PS I reaction centers [7,9], the functional antenna sizes of PS II and PS I may differ significantly [8,14]. However, the question of the chlorophyll composition of the pigment bed of each photosystem has been investigated only through fractionation procedures, and no precise data exist for the in vivo chlorophyll composition of PS I, PS II_α, or PS II_β.

In this report we present the results of spectrophotometric and kinetic analyses on the stoichiometries of the chloroplast photosystems, on the functional light-harvesting chlorophyll antenna size of each reaction center, and on the proposed chlorophyll composition of PS II_α, PS II_β and PS I. Under conditions of green actinic excitation when all Chl $(a + b)$ molecules absorb equally, the relative electron-transport capacity of PS II of spinach thylakoids was greater than that of PS I. The results are discussed in terms of the structural-functional organization of the pigment-protein complexes in the chloroplast thylakoids.

Materials and Methods

Chloroplasts were isolated by grinding leaves of spinach (*Spinacea oleracea* L.) in 50 mM Tricine buffer, pH 7.8, containing 0.4 M sucrose, 10 mM NaCl and 5 mM $MgCl_2$. The slurry was filtered through cheesecloth and chloroplasts were precipitated by centrifugation at 3000 × *g* for 5 min. The pellet was resuspended in a small aliquot of the isolation medium. Chlorophyll concentrations were determined in 80% acetone [16].

Chloroplast fluorescence and absorbance difference measurements were performed with a laboratory-constructed modulated split-beam difference spectrophotometer. The optical path length of the cuvette for the measuring beam was 1.4 mm, and for the actinic beam, 1.0 mm. Actinic excitation was provided by green light of uniform field transmitted by a combination of CS 4-96 and CS 3-69 Corning glass filters. Unless otherwise indicated, the actinic light intensity was 6.5 kerg/cm^2 per s. Green light was selected in order to provide as equal as possible excitation of both Chl *a* and Chl *b* molecules. This contention was tested by measuring the integrated absorption $\int_{510}^{625} A(\lambda)T(\lambda)\mathrm{d}\lambda$ for Chl *a* and Chl *b* in the wavelength region between 510 and 625 nm. $A(\lambda)$ is the specific absorption of a Chl molecule as a function of wavelength λ and $T(\lambda)$ is the transmittance of our CS 4-96 and CS 3-69 Corning glass filters. Using the specific absorption data of Zscheile and Comar [17] for Chl *a* and Chl *b* in ether solution we calculated that the integrated absorbance of Chl *b* in the wavelength region between 510 and 625 nm exceeds that of Chl *a* by 22–28%. Assuming for a moment the extreme condition whereby Chl *b* is associated with PS II only, and if 1 out of 2.8 chlorophyll molecules in the antenna of PS II is Chl *b* (Chl *a*/Chl *b* = 1.8, see Ref. 34), then PS II will absorb 7–9% more excitation than if all of its chlorophyll molecules were Chl *a*. In vivo this effect ought to be moderated significantly because PS I is known to contain its own Chl *b* complement and also because the multiple association of chlorophylls with protein would minimize the sharp spectral differences between Chl *a* and Chl *b* in ether.

The absolute concentrations of PS II and PS I reaction center complexes were estimated from quantitative measurements of the light-induced absorbance changes of Q and P-700, respectively [7]. A relative measure of the antenna size of PS I, PS II_α and PS II_β was obtained from the rate constants of the respective primary photoactivity.

The rate of PS II photochemistry was determined under limiting-light conditions from the analysis of the area over the fluorescence induction curve of DCMU and hydroxylamine-treated chloroplasts [12]. The rate of PS I photochemistry was determined from the analysis of the kinetics of the absorbance change at 700 nm, under the same excitation conditions [18]. To eliminate secondary electron transport to $P700^+$ from plastocyanin, cytochrome *f* and plastoquinone, the chloroplasts were pretreated with potassium cyanide which blocks plastocyanin function [19,20]. Chloroplasts were preincubated for 1 h in the presence of 100 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.

Results

Stoichiometries of the reaction centers of PS II and PS I

Fig. 1 shows the amplitude of the actinic light-induced absorbance change in the red (ΔA_{700}), attributed to the photo-oxidation of P-700, the primary electron donor of PS I, and the amplitude of the absorbance change in the ultraviolet (ΔA_{320}), attributed to the photoreduction of the primary electron acceptor Q of PS II. The concentrations of P-700 and Q were determined from the absorbance changes after making corrections for the flattening effect [21,22]. We estimated that in spinach chloroplasts the ratio of total Chl (*a* + *b*) to P-700 was 590 and the total Chl/Q ratio was about 310, so that the PS II/PS I reaction center ratio (Q/P-700) is about 1.9. It appears, therefore, that spinach chloroplasts contain almost twice as many PS II reaction centers as PS I reaction centers. Such findings have been reported earlier for chloroplasts from a variety of higher plants [9]. However, the implications of such results for the process of linear electron transport have not been fully considered.

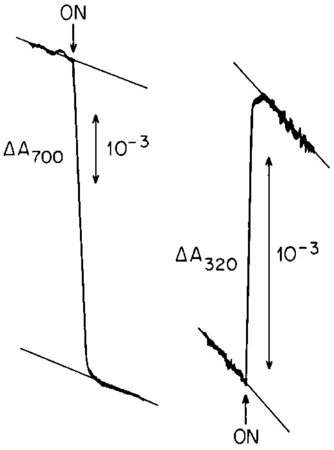

Fig. 1. Actinic light-induced changes in the absorbance of chloroplasts at 700 nm (ΔA_{700}) and at 320 nm (ΔA_{320}). The chloroplasts were suspended in the presence of 2.5 mM sodium ascorbate, 200 μM methyl viologen and 0.2% SDS at a Chl (*a* + *b*) concentration of 300 μM for the ΔA_{700} measurement, and in the presence of 25 μM DCMU and 2.5 mM potassium ferricyanide at 307 μM Chl (*a* + *b*) for the ΔA_{320} measurement. The differential flattening correction factor for the ΔA_{320} measurement was determined according to Ref. 22 and was equal to 1.4.

A thorough understanding of the coordination of the two photosystems in electron transport in higher plant chloroplasts requires, in addition to the stoichiometries of the photosystem reaction centers, the determination of two other important photochemical parameters, i.e., the absolute size of the light-harvesting antenna for each reaction center and the chlorophyll composition of each photosystem. In the present work we provide an evaluation of these parameters in an effort to obtain a closer insight into the chlorophyll composition of the photosystems and on the coordination of the photosynthetic reaction centers in electron transport.

Determination of rate constants

A relative measure of the antenna size of each photosystem was obtained from the rate constant of primary photochemistry under light-limiting conditions. Fig. 2A shows the kinetics of the absorbance decrease at 700 nm (ΔA_{700}) attributed to the photo-oxidation of P-700, and Fig. 2B shows the fluorescence induction curve of DCMU-poisoned chloroplasts, reflecting the rate of Q photoreduction. Since both phenomena are strictly photochemical in nature [18,23], the rate at which they occur depends only on the rate of light absorption by the photochemical pigment beds of the respective reaction center complexes. The light absorption rate by a pigment bed is directly proportional to the incident light intensity and to the number of light-absorbing molecules. Given that the former parameter is constant in our measurements, any difference in the rates of photochemistry of PS II and PS I must be attributed to differences in the light-harvesting antenna size (i.e., functional chlorophyll/reaction center ratio).

The rate constants of the two light-dependent processes were determined from the semilogarithmic plots of the kinetic measurements of ΔA_{700} (Fig. 3A) and from the area over the fluorescence induction curve (Fig. 3B). As reported earlier [14,18], the photo-oxidation of P-700 is a monophasic first-order function of time as illustrated by the single straight line in the semilogarithmic plot of Fig. 3A. The slope of this line defined the rate constant $K_{\text{P-700}}$ which, under our experimental conditions, was approx. 10.0 s^{-1}.

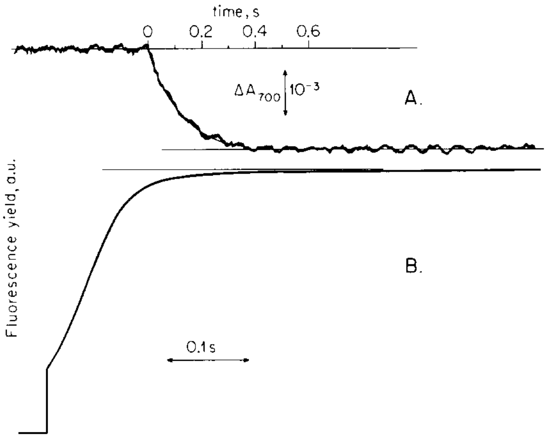

Fig. 2. (A) The time course of the absorbance change at 700 nm monitored with potassium cyanide-poisoned chloroplast in the presence of 200 μM methyl viologen. (B) The fluorescence induction curve of isolated chloroplasts in the presence of 25 μM DCMU and 5 mM hydroxylamine. Chlorophyll concentration was 248 μM.

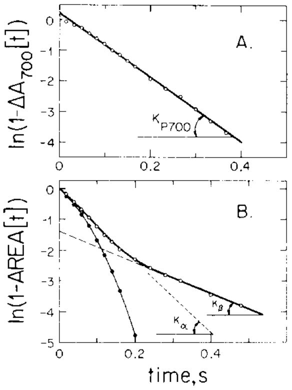

Fig. 3. (A) A semilogarithmic plot of the kinetics of ΔA_{700} showing the monophasic exponential nature of P-700 photo-oxidation. The slope of the straight line defines the rate constant $K_{\text{P-700}}$. (B) A semilogarithmic plot of the kinetics of the area over the fluorescence induction curve (open circles). The slope of the slow linear phase defines the rate constant K_β of Q_β photoreduction. The semilogarithmic plot of the kinetics of Q_α photoreduction (solid circles) deviates from linearity. The rate constant K_α was determined from the slope of the nonlinear curve (solid circles) at zero time.

The photoreduction of Q occurs with biphasic kinetics, revealing in higher plant chloroplasts the existence of two types of PS II reaction center complexes, PS II_α and PS II_β [13,24]. Fig. 3B (open circles) illustrates the biphasic nature of photochemistry at PS II. The slow linear phase, attributed to the photoactivity of PS II_β, is a monophasic first-order function of time, the slope of which defined the rate constant, K_β equal to 4.8 s^{-1}. The fast phase of PS II photoactivity is non-first order and its kinetics are determined by subtracting the slow first-order phase from the overall kinetic phenomenon (Fig. 3B, solid circles). The rate constant K_α of PS II_α was estimated from the slope of the semilogarithmic plot at zero time to be 11.2 s^{-1}. The nonlinear nature of the photoreduction of PS II_α has been attributed to the organization of PS II_α in clusters or aggregates of units sharing the same statistical pigment bed. As seen in Fig. 3B (solid circles) the slope, or the rate constant of PS II_α photoactivity, increases progressively. This occurs as the reaction centers become closed because progressively fewer reaction centers in the statistical pigment of PS II$_\alpha$ receive excitation from the common antenna. The relative ratio of the slope K_α at the end of the process to that at its beginning offers a direct measure of the number of individual PS II$_\alpha$ units forming a cluster. In several such measurements with spinach, we determined that the terminal value of the slope K_α approached 40–50 s^{-1}, i.e., about 4-5-fold its value at zero time (see Fig. 3B, solid circles). A direct interpretation of this phenomenon is that four to five individual PS II$_\alpha$ units aggregate to form a cluster in the partition region of thylakoid membranes. There would be many PS II$_\alpha$ clusters in each membrane partition region, probably equivalent to the EF freeze-fracture particles.

Chlorophyll antenna sizes of PS II$_\alpha$, PS II$_\beta$ and PS I

Analysis of the results of Fig. 3B provides also an estimate of the relative proportion of PS II$_\beta$ reaction centers in the spinach chloroplasts [12]. PS II$_\beta$ accounts for about 25% of the total PS II reaction centers while the remaining 75% is PS II$_\alpha$. Since the overall PS II/PS I reaction center ratio is 1.9, it follows that the reaction center ratios of PS II$_\alpha$/PS I is 1.43 and PS II$_\beta$/PS I is 0.48 (Table I). From these ratios and from the experimentally determined values of the rate constants K_α, K_β and $K_{\text{P-700}}$, the absolute numbers, N_α, N_β and $N_{\text{P-700}}$, of chlorophyll molecules transferring excitation to the reaction centers of PS II$_\alpha$, PS II$_\beta$ and PS I, respectively, were determined. This was implemented by the solution of the following system of equations:

$$\frac{\text{Chl}}{\text{PS I}} = \frac{\text{PS II}_\alpha}{\text{PS I}} N_\alpha + \frac{\text{PS II}_\beta}{\text{PS I}} N_\beta + N_{\text{P-700}} \tag{1}$$

$$K_\alpha = cIN_\alpha \tag{2}$$

$$K_\beta = cIN_\beta \tag{3}$$

$$K_{\text{P-700}} = cIN_{\text{P-700}} \tag{4}$$

where Chl/PS I is the ratio of the total Chl $(a + b)$ per PS I reaction center, I the actinic light intensity and c a proportionality constant depedending on the quantum yield of photochemistry at each photosystem. Although the quantum yield of photochemistry at the three photosystems is not unity, it is generally accepted that it is greater than 0.8 [10,25–27]. For the solution of Eqns. 1–4 we assumed that the quantum yields for photochemistry at PS II$_\alpha$, PS II$_\beta$ and PS I are

TABLE I

PHOTOSYSTEM STOICHIOMETRIES, ANTENNA SIZE AND ELECTRON-TRANSPORT CAPACITY IN SPINACH CHLOROPLASTS

The concentration of the photosynthetic reaction centers was obtained from the amplitude of the absorbance change measurements in the ultraviolet (ΔA_{320}) and red (ΔA_{700}) regions of the spectrum. The antenna size of each photosystem was determined from the rate constant of the respective photochemistry under continuous illumination of limiting intensity. The relative electron-transport capacity of each photosystem was determined as the product of the respective light-harvesting antenna size times the relative concentration of the respective photosystem.

	Photosystem		
	PS II$_\alpha$	PS II$_\beta$	PS I
Relative concentration of reaction centers	1.43	0.48	1.00
Rate constant (s^{-1})	11.2	4.8	10.0
Functional light-harvesting antenna size	N_α 234	N_β 100	$N_{\text{PS I}}$ 209
Relative proportion of total Chl in each photosystem	57	8	35
Relative proportion of total Chl in PS II	88	12	
Relative electron-transport capacity	1.60	0.23	1.00

similar, thereby using the same proportionality constant c. The resulting values of $N_\alpha = 234$, $N_\beta = 100$ and $N_{\mathrm{P}\text{-}700} = 209$ (Table I) are in agreement with those given by Thielen and Van Gorkom [8] for wild-type tobacco. They underscore a basic imbalance in electron-transport capacity between PS II and PS I: not only is there a greater number of PS II reaction centers compared to PS I reaction centers in higher plant chloroplasts, but also the chlorophyll antenna per reaction center appears to be greater overall for PS II than for PS I. The conclusion from these results is that the overall electron-transport capacity of PS II in spinach is significantly greater than that of PS I. A first approximation of the relative electron-transport capacity of each photosystem is obtained by multiplying the relative concentration of each reaction center by its antenna size. Calculation of the electron-transport capacity ratio (C):

$$C = \frac{[\mathrm{PS\ II}_\alpha] N_\alpha + [\mathrm{PS\ II}_\beta] N_\beta}{[\mathrm{PS\ I}] N_{\mathrm{P}\text{-}700}} = 1.83 \qquad (5)$$

indicates that there is an approx. 1.8-times greater relative electron-transport capacity in PS II compared to PS I.

Relative amounts of chlorophyll associated with PS II_α, PS II_β and PS I

Table I summarizes the experimental data on reaction center stoichiometries and functional antenna size for each of the three photosystems. Using the data of Table I, we calculated the relative distribution of chlorophyll amongst the photosystems of spinach thylakoids to be 57% the total chlorophyll in PS II_α compared to 8% in PS II_β and 35% in PS I. Hence there is 7-times as much chlorophyll in PS II_α compared to PS II_β, and about 1.8-times as much chlorophyll in PS II as in PS I (Table I).

Implications for linear electron flow through PS II

The significantly greater relative electron-transport capacity of PS II has important implications for the linear electron-transport process under conditions where all Chl $a + b$ molecules are excited equally. Illumination of chloroplasts with weak continuous green light, for example, would cause turnover of electrons by PS II at a rate approx. 80% faster compared to that of PS I. This would result in the accumulation of electrons in the pool of electron acceptors between the two photosystems, and eventually by Q itself, effectively shutting off a significant portion of PS II. To what extent this phenomenon occurs in nature is not known. However, we verified the validity of the above derivations with isolated chloroplasts. The steady-state oxidation-reduction state of PS II and of the plastoquinone pool was monitored by means of the chlorophyll fluorescence yield under continuous, light-limiting conditions (weak green light). The oxidation-reduction state of P-700 was measured independently. Fig. 4 shows the fluorescence induction curves of isolated chloroplasts in the absence of any artificial electron acceptor (control), or with 200 μM methyl viologen or 1 mM potassuim ferricyanide. All samples were illuminated by green light of the same low intensity (light-limiting conditions). Illumination of control chloroplasts caused an increase in the variable fluorescence yield (F_v), to approx. 3-times the yield of the nonvariable fluorescence emission (F_0). The variable fluorescence yield increase observed with control chloroplasts was approx. 75% of that measured in the presence of DCMU under identical illumination conditions, suggesting that ap-

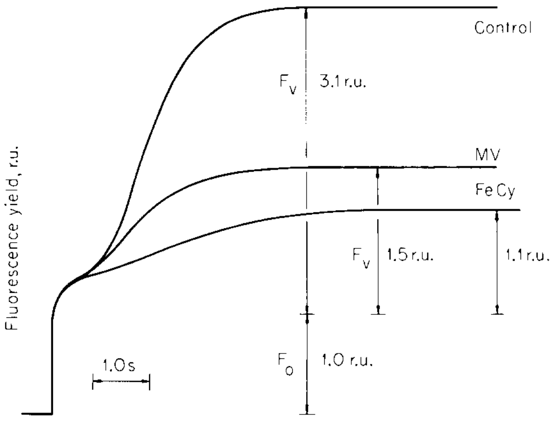

Fig. 4. The fluorescence induction curve of isolated chloroplasts in the absence of any artificial electron acceptor (Control), in the presence of 200 μM methyl viologen (MV), and in the presence of 1 mM potassium ferricyanide (FeCy). The amplitude of the variable fluorescence yield increase (F_v) was measured on the basis of the amplitude of the nonvariable fluorescence emission ($F_0 = 1.0$ relative units).

prox. 75% of PS II reaction centers were closed due to the accumulation of electrons on Q. This accumulation of electrons between the two photosystems is caused by the inability of the Mehler reaction [28] to sustain a rapid flow of electrons from the reducing side of PS I to molecular oxygen (half-times of 150 ms). Such a limitation is alleviated by methyl viologen which removes electrons from the PS I Fe-S centers with a half-time of less than 1 ms [29].

Under our light-limiting experimental conditions, the rate constant of PS I photoactivity was 10.0 s^{-1} (see Fig. 3A) which is equivalent to a half-time of 100 ms, i.e., significantly slower than the removal of electrons by methyl viologen. Fig. 4 shows there was still a significant increase in variable fluorescence yield in the presence of methyl viologen, equal to about 36% of the total variable fluorescence. The variable fluorescence yield increase reflects the accumulation of Q^- but it is not directly proportional to it. Melis and Schreiber [30] presented an experimental calibration curve of Q^- accumulation as a function the relative variable fluorescence yield increase. From the nonlinear relationship of Q^- versus F_v [30], we determined that a variable fluorescence yield increase of 36% reflects the inhibiton of about 45% of PS II reaction centers due to accumulation of electrons on Q under steady-state conditions. It is suggested that in spite of the efficient removal of electrons from the donor side of PS I, 45% of the PS II reaction centers, on average, became closed at the steady state. This loss of 45% of the PS II electron-transport capacity was apparently precipitated by the greater turnover rate of PS II than PS I under our experimental conditions, and the consequent accumulation of electrons between the two photosystems. Fig. 4 also shows that, in the presence of 1 mM potassium ferricyanide, electrons generated by PS II are not removed efficiently from the electron acceptors between the two photosystems. The increase in variable fluorescence observed under these conditions is approx. 27% of the total variable fluorescence, pointing to inhibition of about 40% of the PS II reaction centers.

Our results suggest that potassium ferricyanide is a rather poor acceptor of electrons from the reducing side of PS II [31,32], even when the rate of PS II turnover is light-limited, and as low as 11.2 electrons/reaction center per second (see Fig. 3B, solid circles). We will present evidence below that potassium ferricyanide is an efficient PS I electron acceptor.

Implications for linear electron flow through PS I

The results presented in Fig. 4 clearly suggest that PS I does not have the capacity to process all the electrons transferred by PS II and that this imbalance in the electron-transport rate of the two photosystems is reflected by the accumulation of electrons in the pool of intermediate acceptors and finally by Q, thereby shutting off a significant portion of PS II reaction centers. This phenomenon resulted in the variable fluorescence yield increase shown in Fig. 4 for chloroplasts in the presence of methyl viologen or potassium ferricyanide. For chloroplasts in the presence of methyl viologen, we estimated that about 45% of all PS II reaction centers became closed at steady state. Since the overall PS II/PS I electron-transport capacity ratio was approx. 1.83 (see Eqn. 5), the inhibition of 45% of PS II would effectively reduce the operational electron-transport capacity ratio to about 1.0, suggesting that at steady state there is a balanced overall flow of electrons between the two photosystems. How this inhibition is manifested at the membrane level among the separate PS II_β units and the aggregates of PS II_α units is not known at present. It is conceivable that entire PS II_α aggregate units that are located at a greater distance from the cytochrome b_6-f complex would remain closed, under the above experimental conditions, since such units would require a greater average shuttling distance by plastoquinone in the transport of electrons from Q_α to the Fe-S Rieske center.

On the basis of the above considerations one would expect all PS I reaction centers to remain fully reduced under our experimental conditions, since the supply of electrons from PS II is greater than the capacity of PS I to process them. This should hold irrespective of the electron acceptor used, methyl viologen or ferricyanide. Fig. 5 (DCMU) shows that upon illumination of isolated and DCMU-poisoned chloroplasts with weak green light, P-700 becomes fully photo-oxidized. Fig. 5 (MV) also shows that illumination in the presence

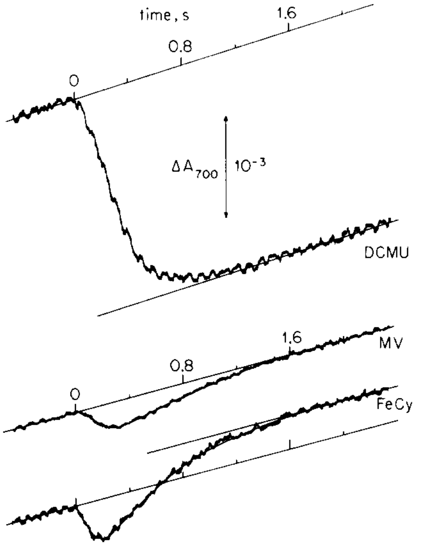

Fig. 5. Absorbance change kinetics of chloroplasts (210 μM Chl) in the presence of 25 μM DCMU, 200 μM methyl viologen (MV) and 1.0 mM potassium ferricyanide (FeCy). The actinic illumination was turned on at zero time. The samples also contained 50 μM gramicidin.

of methyl viologen, but in the absence of DCMU, causes a short-lived transient oxidation of only part of the P-700 pool, and that at steady state the P-700 pool becomes fully reduced again. Clearly, the transient oxidation of P-700 occurs because of the time required for PS II electrons to reach PS I reaction centers. The amplitude of the P-700 transient oxidation accounts for only a small portion of the total P-700 signal. It varied in different preparations, occasionally being negligibly small. Fig. 5 (FeCy) shows that a transient oxidation of P-700 was detected also in the presence of 1 mM potassium ferricyanide but, unlike with methyl viologen, the signal crossed the baseline showing an overall small positive absorbance change at steady state. The addition of 1 mM ferricyanide apparently caused the oxidation of a small portion of P-700 in the dark. This chemical oxidation was fully reversed in the light, in spite of the high ferricyanide concentration present.

Electron acceptor efficiency of methyl viologen and ferricyanide

The results of Fig. 5 complement those of Fig. 4 and support the interpretation of a greater PS II electron-transport rate compared to PS I. They suggest also that potassium ferricyanide is a very poor electron acceptor for PS II, even at concentrations as high as 1 mM. We tested the effectiveness of potassium ferricyanide as a PS I electron acceptor using plastocyanin-inhibited chloroplasts. The kinetics of P-700 photo-oxidation (Fig. 6) and the respective semilogarithmic plots (Fig. 7) are shown for chloroplasts in the absence of any artificial electron acceptor, or in the presence of methyl viologen (100 μM) or in the presence of potassium ferricyanide (200 μM). In the absence of any electron acceptors, the P-700 photo-oxidation kinetics are significantly slower, and they deviated from exponentiality due to inef-

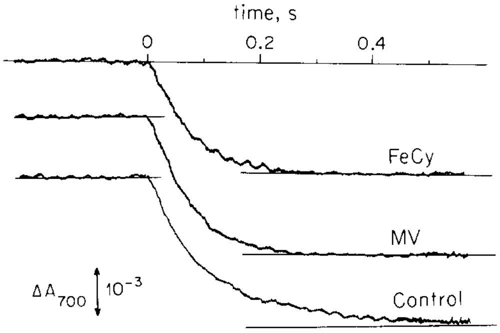

Fig. 6. Absorbance change kinetics of potassium cyanide-inhibited chloroplasts (220 μM Chl) in the absence of any artificial electron acceptor (Control), in the presence of 100 μM methyl viologen (MV), or in the presence of 100 μM potassium ferricyanide. Actinic light intensity of 9.3 kerg/cm^2 per s.

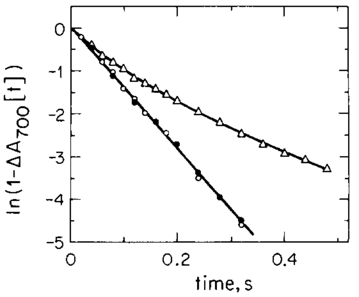

Fig. 7. A semilogarithmic plot of the kinetics of ΔA_{700} from Fig. 6. Chloroplasts in the absence of any artificial electron acceptor (△), in the presence of 200 μM ferricyanide (●) or in the presence of 100 μM methyl viologen (○). Note the deviation of the kinetics from linearity in the absence of any artificial electron acceptor.

ficient electron removal from the reducing side of PS I by molecular oxygen (Mehler reaction). In the presence of 100 μM methyl viologen, this limitation is alleviated and the P-700 photo-oxidation kinetics became an exponential function of time as predicted [18]. Interestingly, the same effect is brought about by the addition of a small amount (200 μM) of potassium ferricyanide (Fig. 7, solid circles), suggesting that potassium ferricyanide is as efficient a PS I electron acceptor as methyl viologen.

Discussion

The stoichiometric measurements and the kinetic analyses of PS II_α, PS II_β and PS I presented in this work have clearly established that the relative electron-transport capacity of PS II is greater than that of PS I. This conclusion should be qualified by the observation that the electron-transport capacity of a chloroplast photosystem depends on three main photochemical parameters: (1) the relative concentration of that photosystem, (2) the absolute size of the light-harvesting chlorophyll antenna per reaction center, and (3) the chlorophyll composition of each photosystem. In the present work we have calculated that the overall ratio of PS II/PS I reaction centers is 1.9 in spinach chloroplasts and that the reaction center ratio of PS II_α/PS I is 1.4 and that PS II_β/PS I is 0.5. Furthermore, we have presented evidence that the number of Chl $(a+b)$ molecules associated with PS II_α ($N_\alpha = 234$) is greater than that of PS I ($N_{\text{P-700}} = 210$) with PS II_β having a significantly smaller antenna size ($N_\beta = 100$, Table I). The third photochemical parameter, namely, the chlorophyll composition of each photosystem was eliminated as a variable in our present derivations, by working with green actinic illumination which provided equal excitation of both Chl *a* and Chl *b* molecules. Naturally, this constraint would not apply under physiological conditions.

On the basis of our biophysical data and that on the composition of Chl-proteins derived from chloroplast fractionation studies, we have calculated the pigment composition of PS II_α, PS II_β and PS I as follows. It has been demonstrated that purified PS II particles isolated from spinach grana thylakoids following mild Triton X-100 treatment [33] lack both PS I and PS II_β [34]. These PS II particles which are active in O_2 evolution have a Chl *a*/Chl *b* ratio of about 1.7 and a Chl/Q ratio of 230 [34]. Hence, our kinetic determination of $N_\alpha = 234$ is in excellent agreement with the measured Chl/Q ratio of PS II particles. The light-harvesting antenna of PS II_α is composed of the core PS II reaction center complex, which includes the P-680-Chl *a*-β-carotene-proteins of PS II, and the surrounding Chl *a*/*b*-proteins of the main light-harvesting complex (LHC) [35,36]. Assuming a Chl *a*/Chl *b* ratio of 1.25 for the Chl *a*/*b*-proteins of the PS II-LHC [35,36] we calculate that PS II_α contains a core of 40 Chl *a* surrounded by 106 Chl *a* and 84 Chl *b* molecules. Thus, the ratio of the total chlorophyll of the Chl *a*/*b*-proteins to that of the core reaction center complex of PS II (190 : 40 = 4.75, see Table II) is also in good agreement with the value determined from the gel-electrophoretic analysis of the Chl-proteins of spinach thylakoids (65 : 14 = 4.7) [37].

Stroma thylakoids which have no PS II_α and contain only PS I and PS II_β have been isolated from spinach thylakoids fragmented by Yeda press treatment [15]. The Chl *a*/Chl *b* ratio of this membrane fraction is 5.9, suggesting a marked depletion in the main Chl *a*/*b*-proteins of the PS II-LHC. Indeed, gel-electrophoretic analysis showed that 70% of the total Chl of stroma thylakoids is associated with the PS I complex, 5% with the PS II complex and 17% with the Chl *a*/*b*-proteins of the main light-harvesting complex [37].

The exact Chl-protein composition of PS I has not yet been determined. However, it is now clear that the undissociated PS I complex has its own complement of Chl *b*, noncovalently bound to intrinsic polypeptides in the 27–20 kDa range, quite distinct from the apoproteins of the main Chl *a*/*b*-proteins (30–27 kDa) [38–41]. Several PS I complexes have been isolated: a Triton X-100-PS I complex (Chl *a*/Chl *b* of 6.0) with an antenna size of 184, and a gel complex CP 1a (Chl *a*/Chl *b* of 11) with an antenna size of 120 [38]. Mullet et al. [39–41] showed that the core reaction center complex of PS I, with an antenna unit size of about 40 Chl *a* was depleted in the 25–20 kDa proteins associated with the peripheral LHC of PS I. We suggest that PS I consists of a core of 40 Chl

TABLE II

CHLOROPHYLL COMPOSITION OF SPINACH THYLAKOID PHOTOSYSTEMS

The three chloroplast photosystems are localized in different areas of the thylakoid membrane [15] and show different chlorophyll composition. The total antenna size was determined from stoichiometric and kinetic analysis of the absorbance change measurements in the ultraviolet (ΔA_{320}) and red (ΔA_{700}) regions of the spectrum. The estimation of the core reaction center complex of PS II_α was based on the Chl *a*/*b* LHC composition of PS II_α while the estimation of the core reaction center complex of PS I and PS II_β was based on the data given in Refs. 39–41 and 8, respectively.

	Photosystem		
	PS II_α	PS I	PS II_β
Proposed location	Grana partition membranes	Stroma-exposed thylakoids (intergrana membranes)	Stroma-exposed thylakoids (end-grana thylakoids)
Antenna size	230	210	100
Chl *a*/Chl *b*	1.73	6.0	5.6 [a]
Core reaction center complex	40 Chl *a* [a]	40 Chl *a* [a]	40 Chl *a* [a]
	LHC-II_α	LHC-I	LHC-II_β
Chl *a*/*b*-proteins	106 Chl *a*	140 Chl *a* [a]	45 Chl *a*
	84 Chl *b*	30 Chl *b*	15 Chl *b*
Chl *a*/Chl *b*	1.25	4.7 [a]	3.0 [a]
LHC/core complex	4.75 [a]	4.25 [a]	1.50 [a]
Total Chl *b* in each photosystem	36.5% Chl *b* [a]	14.3% Chl *b* [a]	15% Chl *b* [a]

[a] Estimated values.

a surrounded by peripheral Chl *a*/*b*-proteins containing 140 Chl *a* and 30 Chl *b* molecules (Chl *a*/Chl *b* ratio of 4.7, see Table II).

The Chl *a*/Chl *b* ratio of PS II_β is unknown, but if stroma thylakoids (Chl *a*/Chl *b* ratio of 5.9) contain only PS I and PS II_β, and if PS I itself is assumed to have a Chl *a*/Chl *b* ratio of 6.0, then that of PS II_β should be about 5.6 (Table II). With an antenna size of 100 molecules, PS II_β would have 15 Chl *b* and 85 Chl *a* molecules. However, since Thielen and Van Gorkom [8] found a minimal antenna unit size of 40 for PS II_β in tobacco mutants, and this PS II_β had no Chl *b*, we propose that PS II_β contains a core of 40 Chl *a* molecules surrounded by 45 Chl *a* and 15 Chl *b* molecules, i.e., the PS II_β Chl *a*/*b*-proteins should have a Chl *a*/Chl *b* ratio of 3.0 (Table II).

In short, the chlorophyll content of the appressed membranes is about 57% and that of the nonappressed regions is about 43% of the total [42]. A comparison of the calculated content of Chl *a* and Chl *b* in the two membrane regions (Table II) shows that the total amounts of Chl *a* are roughly comparable. In contrast, there is a 3.8-fold increase in Chl *b* in the appressed membranes, probably due to the need to extend the spectral window of absorption as light penetrates through the high-density membranes of the grana stacks.

An explanation of the heterogeneity in the composition, organization and function of the photosystems in photosynthetic membranes may be found by considering two variable factors that occur under natural plant growth conditions: (a) the different chlorophyll composition of each photosystem and (b) the significant attenuation of sunlight through the various cell layers of a leaf. In the first category, one should consider the much greater Chl *b* content of PS II_α compared to either PS I or PS II_β which appear to have rather similar Chl *b* contents (Table II). PS II_β and PS I also have more of the longer wavelength forms of Chl *a* [43]. Although Chl *b* significantly widens the useful spectral window by extending the absorbance range of the system into the green to 475 nm and 650 nm in the red region, Chl *b* may not be as effective at light absorption as Chl *a*. The extinction coefficients of Chl *a* and Chl *b* when complexed to protein are not known, but it is likely that the integrated absorption of Chl *b* is very much less than that of Chl *a*, especially in the red region. Since the large antenna size of PS II_α ($N_\alpha = 234$) is partly compensated for by its enhanced Chl *b* content compared to either PS II_β or PS I, the overall imbalance of Chl molecules in PS II compared to PS I is not as great as it appears at first sight.

In the second category, given the existence of several layers of chloroplasts in a leaf of spinach, then only the uppermost chloroplast layers of the palisade parenchyma are exposed to full sunlight. Lower layers of chloroplasts in the leaf are partially shaded and receive excitation that is greatly depleted in the blue and red spectral components. In turn, this effect is greater in the grana stacks compared to stroma thylakoids. Consequently, the attenuation of sunlight through the leaf would advantage PS I which can make more efficient use of longer wavelength excitation ($\lambda > 680$ nm) than PS II. This phenomenon is expected to favor electron transport through PS I and thus work towards balancing further electron flow between the two photosystems. This interpretation is supported by the structural and functional properties of chloroplasts from obligate shade species in which proximal PS II electron-transport capacity is 3–4-fold greater than that of PS I [9]. Obligate shade species normally live in the lower canopy of forests where they receive excitation energy from sunlight after it is filtered through the upper canopy of the forest tree (apart from direct sunflecks). Such excitation is known to be very significantly enriched in PS I excitation (over 90% of the quantum flux density is greater than 690 nm [44]). A similar situation of differential light attenuation also exists in the microenvironment of the leaves of sun-adapted species, especially since the upper surface of sun-adapted leaves contain two layers of palisade cells, normally packed with chloroplasts [44,45].

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