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Functional properties of Photosystem II_β in spinach chloroplasts

Anastasios Melis

Division of Molecular Plant Biology, 313 Hilgard Hall, University of California, Berkeley, CA 94720 (U.S.A.)

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The activity of Photosystem (PS) II_β was monitored for the first time in the absence of added herbicides in isolated chloroplast samples and in leaves of spinach in vivo. The new approach was implemented following identification of the initial chloroplast fluorescence rise from F_0 to F_{p1} (Forbush, B. and Kok, B. (1968) Biochim. Biophys. Acta 162, 243–253) as the variable fluorescence yield controlled by PS II_β. Evidence is presented indicating the absence of the intermediate plastoquinone pool from the thylakoid membrane in which PS II_β is localized. A two-step developmental process for PS II assembly and grana formation is proposed. According to this working hypothesis, PS II_β is the precursor form of PS II_α, structurally and functionally complete except for the absence of the peripheral chlorophyll *a/b* light-harvesting antenna and the complement of the plastoquinone pool. It is postulated that addition of these two hydrophobic components converts PS II_β into PS II_α and initiates the incorporation of the thylakoid membrane into grana.

Introduction

The concept of PS II heterogeneity was first introduced about 8 years ago in order to interpret the biphasic nature of primary System II photoactivity [1,2]. Different aspects of this heterogeneity have been addressed by several investigators, and it is now believed that PS II occurs in the form of two structural-functional configurations, termed PS II_α and PS II_β. The two types of PS II reaction center complexes differ in both the effective light-harvesting Chl antenna size [2,3] and their location in the chloroplast membrane: only PS II_α possessed the full complement of the peripheral Chl *a/b* LHC II, and was localized in the membrane of the grana partition region [3,4]. On the other hand, PS II_β possessed only a small complement of the LHC II [3,5,6] and, in addition, it has been isolated along with a stroma-thylakoid fraction after Yeda press treatment of chloroplasts [3].

The functional significance of the differentiation of System II into PS II_α and PS II_β in higher plant chloroplasts is unknown. The major PS II_α with its larger antenna size and enhanced Chl *b* content is the predominant source of electrons for linear electron transport and noncyclic photophosphorylation, as discussed in earlier publications [3,6]. In the present work evidence is presented to show the absence of the intermediate plastoquinone from the thylakoid membrane in which PS II_β resides. A new approach is presented for the detection of PS II_β activity in isolated chloroplasts in the absence of herbicides and in leaves of spinach in vivo. We propose a developmental relationship between PS II_α and PS II_β in higher plant chloroplasts.

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4'-dichlorophenyl)-1,1-dimethylurea; PS, Photosystem; Q, primary electron acceptor of PS II; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; DMQ, dimethylbenzoquinone; TMQ, tetramethylbenzoquinone; LHC II, peripheral Chl light-harvesting complex of PS II; Cyt, cytochrome.

Materials and Methods

Spinach plants (*Spinacea oleracea* L.) were grown in the greenhouse on half-strength Hoagland nutrient solution. Chloroplasts were isolated by grinding freshly harvested leaves for 10 s in a Waring blender in a medium containing 0.4 M sucrose/10 mM NaCl/5 mM $MgCl_2$/50 mM Tricine (pH 8.0). The slurry was filtered through miracloth, and chloroplasts were precipitated by centrifugation at $5000 \times g$ for 5 min. The pellet was resuspended in isolation buffer at a Chl concentration of about 500 μM using a Wheaton homogenizer.

Hydroxylamine-treated chloroplasts were obtained by incubating an aliquot of the chloroplast suspension in the presence of 20 mM NH_2OH for 15 min. Following this incubation, the chloroplasts were washed twice in the isolation buffer in order to remove all hydroxylamine from the solution and finally resuspended in the same buffer medium at a Chl concentration of about 500 μM. Such treatment resulted in the complete loss of oxygen evolution capacity by the spinach chloroplasts. All operations described above were carried out in dim light at 0°C. Chl concentrations were determined in 80% acetone using the procedure described by Arnon [7]. Intact spinach leaf discs having a 7 mm diameter were taken from the midrib-free area of the leaf. They were floated at room temperature on a 10 mM $NaHCO_3$ (pH 6.7) solution in the dark for 20 min before use.

Chloroplast and leaf fluorescence kinetics were measured with an apparatus previously described [8]. Excitation light was provided in the green region of the spectrum by a combination of CS 4-96 (broad band blue) and CS 3-69 (yellow cut-off) Corning filters. The actinic light intensity was set at 10 $W \cdot m^{-2}$. The photomultiplier tube was shielded from the actinic light by a combination of CS 2-64 (red cut-off) Corning and a Balzers 690 nm interference filters. Signal recovery and processing was implemented by a Hewlett-Packard 3437A digital voltmeter interfaced with an on line HP 86B computer. The kinetic analysis of the data, such as integration of the area over the fluorescence induction curves and the semilogarithmic analysis were performed by the computer. The results were plotted on a HP 7475A plotter.

Results

It was originally recognized by Forbush and Kok [9] that the variable fluoresence kinetics of isolated and dark-adapted chloroplasts consisted of a small initial rise of fluorescence from the non-variable level F_0 to the intermediate plateau level F_{p1} which was then followed by the slower but more pronounced fluorescence yield increase to level F_{max} (see Fig. 1 of Ref. 9). A similar presentation is given in Fig. 1 (Control) of this work. The fluorescence increase from the intermediate plateau level F_{p1} to F_{max} is a manifestation of the plastoquinone pool photoreduction and of the ensuing electron accumulation on the primary electron acceptor Q of PS II. As such, it is largely prevented by the addition of potassium ferricyanide which drains electrons from plastohydroquinone. Surprisingly, the initial rise from F_0 to F_{p1} is not affected by the presence of ferricyanide in the chloroplast suspension medium [9], shown in Fig. 1 (FeCN). A similar result was obtained with NH_2OH-treated chloroplasts where the photoreduction of the plastoquinone did not occur, due to the loss of H_2O-splitting activity, but the manifestation of the initial fluorescence rise from F_0 to F_{p1} remained unaffected, shown in Fig. 1 (NH_2OH).

In DCMU-poisoned chloroplasts, the oxidation of Q^- by plastoquinone is fully inhibited and, upon illumination, a prompt fluorescence increase

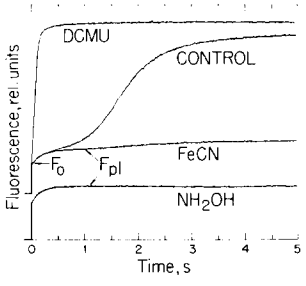

Fig. 1. Fluorescence induction traces of isolated spinach thylakoid membranes (Control) suspended in the presence of 20 μM DCMU or 2 mM potassium ferricyanide (FeCN). Also shown is the trace of hydroxylamine-treated chloroplasts suspended in the absence of any added acceptors (NH_2OH). The actinic light came on at zero time. The Chl concentration of the samples was 100 μM. Note the initial variable fluorescence rise from F_0 to the intermediate plateau level F_{p1}.

from F_0 to F_{max} is observed, shown in Fig. 1 (DCMU). Fluorescence induction kinetics in the presence of DCMU are biphasic, however. The fast non-exponential α component reflects the photoactivity of PS II_α and the slower exponential β component reflects the photoactivity of PS II_β [1–3,5,6,10]. A visual comparison of the kinetic traces shown in Fig. 1 (DCMU, FeCN and NH_2OH) suggested to us that the initial fluorescence rise from F_0 to F_{p1} (FeCN and NH_2OH traces) might be identical to the slower β component in the fluorescence kinetics of the DCMU trace. Therefore, we set out to investigate this possibility.

On a faster time scale, Fig. 2 compares the fluorescence induction kinetics of hydroxylamine-treated (NH_2OH), ferricyanide-added (FeCN) and DCMU-poisoned chloroplasts. To improve the clarity of presentation, the apparatus gain was increased 3× for the NH_2OH and FeCN traces relative to that of DCMU. It is observed that the fast sigmoidal α component dominated the fluorescence induction kinetics in the presence of DCMU, but it is absent both from the NH_2OH and FeCN traces. The latter two are monophasic exponential functions of time. The above contention is verified from the semilogarithmic plot of the areas over the three fluorescence induction traces, shown in Fig. 3. It is observed that the rate (slope) of Q photoreduction in the FeCN and NH_2OH samples is essentially identical to that of the β-phase in the presence of DCMU (K_β). Table I compares the rate constant of the initial fluorescence rise from F_0 to F_{p1}, obtained under different experimental conditions, with that of K_β obtained from the slow phase in the presence of DCMU. Under identical experimental conditions we determined a rate of light absorption by PS II_α, $K_\alpha = 12\ s^{-1}$. Table I also compares the amplitude of the initial fluorescence rise from F_0 to F_{p1}, obtained under different experimental conditions, with that of $F_{v\beta}$ obtained from the slow fluorescence kinetic component in the presence of DCMU. In summary, both the rate and the fluorescence yield of the initial rise from F_0 to F_{p1} accurately match the corresponding parameters of PS II_β. As a consequence, we propose that the initial fluorescence rise from F_0 to F_{p1} in non-poisoned chloroplasts is a manifestation of the photochemical activity of PS II_β. Table I further compares the fraction of PS II designated as PS II_β (determined from the biphasic kinetics it is about 25% of the total) with the fraction of Q that remains stably photoreduced in NH_2OH-treated chloroplasts suspended in the presence of ferricyanide but in the absence of DCMU (390/1520 = 0.256).

In order to eliminate the possibility that both the functional expression of PS II_β in the presence of DCMU and the initial fluorescence rise from F_0

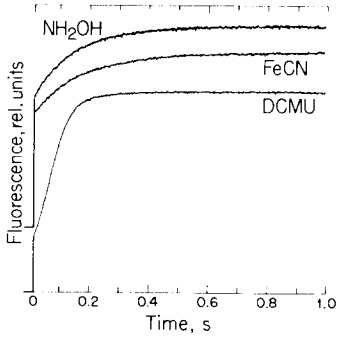

Fig. 2. Fluorescence induction traces of isolated spinach thylakoid membranes suspended in the presence of 20 μM DCMU or 2 mM potassium ferricyanide (FeCN). Also shown is the fluorescence induction trace of hydroxylamine-treated chloroplasts suspended in the absence of any added acceptors (NH_2OH). Compare the sigmoidal and biphasic trace in the presence of DCMU with the monophasic and exponential trace obtained in the presence of FeCN and with NH_2OH-treated chloroplasts. The apparatus gain was set to 3× for the NH_2OH and FeCN traces compared to 1× for the DCMU sample.

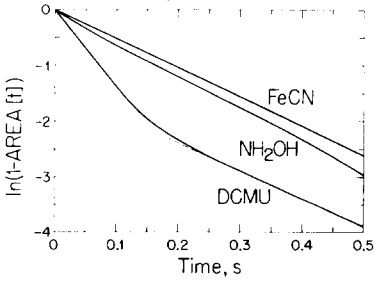

Fig. 3. Semilogarithmic computer plot of the area over the fluorescence induction of the traces shown in Fig. 2. Compare the slopes of the monophasic linear traces (FeCN, NH_2OH) with the slow β component in the presence of DCMU. The intercept of the dashed line with the ordinate at zero time defined the fractional contribution of PS II_β in the area over the fluorescence yield of DCMU-poisoned chloroplasts.

TABLE I

FUNCTIONAL PARAMETERS OF PS II_β IN SPINACH CHLOROPLASTS

Isolated thylakoid membranes were treated as described in Materials and Methods. The rate of light absorption by PS II_β (K_β) was measured from the slope of the semilogarithmic plots shown in Fig. 3. The yield $F_{v\beta}$ of the variable fluorescence controlled by PS II_β was calibrated with respect to the non-variable yield F_0. $F_{v\beta}$ also represents the yield of the initial fluorescence rise from F_0 to F_{p1}. The contribution of PS II_β to the total area over fluorescence yield was estimated from the intercept of the slow β phase in the semilogarithmic plot of Fig. 3 (DCMU sample only). The concentration of the primary electron acceptor Q of PS II was determined from the amplitude of the absorbance change at 320 nm [6].

Chloroplast sample	K_β (s^{-1})	$\frac{F_{v\beta}}{F_0}$	[PS II_β] (%)	$\frac{Chl}{Q}$
FeCN-added	5.1	0.54	–	–
NH_2OH-treated	5.8	0.52	–	1520 [a]
DCMU-poisoned	5.2	0.38 0.52 [b]	25	390
in vivo (leaf)	–	0.52	–	–

[a] Determined with NH_2OH-treated chloroplasts in the presence of 2.0 mM ferricyanide.

[b] Estimated by using the F_0 yield value of control chloroplasts (– DCMU).

to F_{p1} are artifacts created during the chloroplast isolation procedure, we tested for the presence of the initial rise from F_0 to F_{p1} in dark-adapted intact leaves from freshly harvested spinach. Fig. 4 shows, on a slow time scale, the initial rise from F_0 to F_{p1} preceding the large fluorescence yield increase associated with the photoreduction of the plastoquinone pool. Fig. 4 also shows, on an expanded time scale, the kinetic pattern of the F_0-to-F_{p1} transition which appears identical to that shown in Fig. 2 (NH_2OH, FeCN) both in terms of amplitude and exponential kinetics. It must be concluded, therefore, that the slow fluorescence kinetic component attributed to the photoactivity of PS II_β is a basic property of isolated spinach chloroplasts and of chloroplasts in vivo. A similar kinetic component was detected earlier by Joliot in whole green alga cells [11].

It must be noted that, although the $F_{v\beta}/F_0$ ratio was independent of the chloroplast treatment and of the kinetic method used in its estimation (i.e., presence of ferricyanide, hydroxylamine

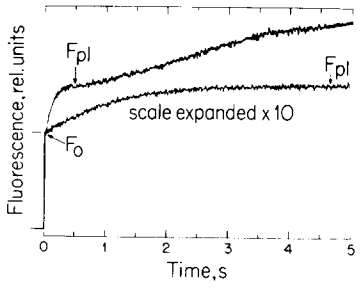

Fig. 4. Fluorescence induction traces obtained with a dark-adapted spinach leaf disc under in vivo conditions. The top trace was obtained upon the first illumination of the leaf disc. The lower trace was obtained on an expanded time scale with the same leaf disc after a 5 min dark recovery. Note the F_0-to-F_{p1} transition in both fluorescence yield traces.

treatment, DCMU inhibition or from the leaf in vivo), we encountered variations of this ratio in different samples. Such variations were in the range of $0.4 < F_{v\beta}/F_0 < 0.8$, and their physiological significance will be discussed in a separate presentation.

The function of PS II_β in the absence of DCMU

The initial fluorescence rise from F_0 to F_{p1} occurs due to the prompt reduction of the primary electron acceptor Q of PS II_β, and is the consequence of the inability of PS II_β to donate electrons to secondary electron acceptors on a fast time scale [9]. The apparent inability of ferricyanide to prevent electron accumulation on the primary electron acceptor Q of PS II_β could be due to the absence of plastoquinone from the thylakoid membrane in which PS II_β is localized. We reasoned that ferricyanide is a poor PS II electron acceptor, even at high concentrations [6,12] and that perhaps more lipophilic electron acceptors could efficiently reoxidize this small pool of Q^-, thereby preventing the initial rise from F_0 to F_{p1}. We found that dimethyl and tetramethyl benzoquinone fulfilled this function and the fluorescence yield remained suppressed in the presence of such artificial electron acceptors [12]. Fig. 5A shows the effect of dimethyl benzoquinone (DMQ) on the amplitude of the initial fluorescence rise from F_0 to F_{p1}. The amplitude of the F_0 to F_{p1} rise diminished proportionally with the concentration

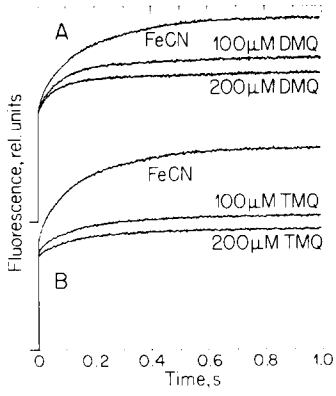

Fig. 5. (A) Fluorescence induction traces of isolated spinach thylakoid membranes suspended in the presence of 2 mM potassium ferricyanide (FeCN), 100 μM or 200 μM dimethyl benzoquinone (DMQ). The actinic light came on at zero time. (B) Fluorescence induction traces of spinach thylakoids suspended in presence of 2 mM ferricyanide (FeCN), 100 μM or 200 μM tetramethyl benzoquinone (TMQ). The Chl $(a + b)$ concentration of all samples was about 100 μM. Note the negligible static quenching of excitation, as expressed on the yield of the non-variable fluorescence F_0, induced by DMQ ($\approx 5\%$) and the more pronounced static quenching of excitation induced by TMQ ($\approx 15\%$).

of the DMQ added. Fig. 5B shows the more pronounced effect of tetramethyl benzoquinone (TMQ) on the yield of the initial fluorescence rise from F_0 to F_{p1}, attributed to the greater solubility of TMQ in the thylakoid membrane lipid bilayer and/or accessibility of TMQ to the quinone binding site of PS II_β. It must be concluded, therefore, that PS II_β will transfer electrons to suitable artificial electron acceptors (added quinones), but not to potassium ferricyanide and clearly not to plastoquinone under physiological conditions.

Previous work from this and other laboratories has revealed that herbicides, in addition to blocking the Q-PQ interaction, interfere with the process of excitation energy transfer and fluorescence emission [13,14]. This is especially pronounced at high herbicide concentrations and it affects PS II_β sooner than it affects PS II_α. Having now established a method for monitoring PS II_β activity in the absence of any herbicides, i.e., from the initial fluorescence rise from F_0 to F_{p1}, we tested a basic property of PS II_β, previously established in the presence of DCMU, i.e., the apparent absence of connectivity among PS II_β units [2,3,6]. The rationale for undertaking this experimentation is the ability now to test for the functional properties of PS II_β from the initial fluorescence rise from F_0 to F_{p1}, thus avoiding both the use of a herbicide and the kinetic separation of PS II_β activity from the dominant PS II_α activity.

Fig. 6A shows a family of fluorescence induction traces obtained in the presence of DCMU with dark-adapted chloroplasts (D) and with preilluminated chloroplasts following a partial restoration of the fluorescence induction (restoration for 1, 5 and 30 s is shown). As already reported earlier [15–17], the induction kinetics of the partially restored areas are considerably faster than those of dark control, reflecting cooperativity of excitation transfer among PS II_α units, i.e., 3 to 4 PS II_α units

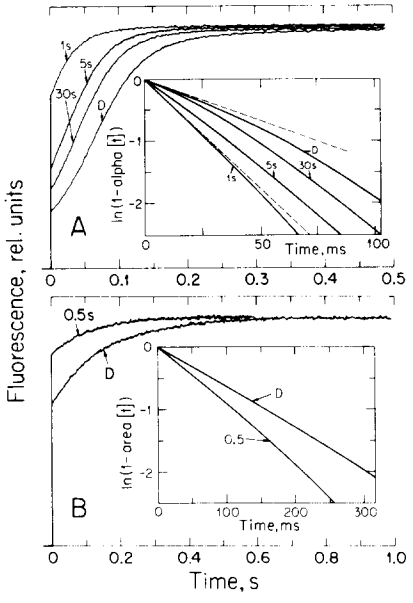

Fig. 6. (A) A family of fluorescence induction traces obtained with DCMU-poisoned chloroplasts that were either dark-adapted (D) or preilluminated for 2 s followed by a partial dark recovery for 1, 5 and 30 s, respectively, before the registration of the fluorescence induction kinetics. Inset: semilogarithmic computer plot of the fast α component of the area of the fluorescence induction traces shown in (A). Note the decreasing initial slope (K_α) as the dark recovery progresses. (B) Fluorescence induction traces of isolated spinach thylakoids suspended in the presence of 2 mM potassium ferricyanide that were either dark-adapted (D) or preilluminated for 2 s followed by a partial dark recovery for 0.5 s before the registration of the fluorescence induction kinetics. Inset: semilogarithmic computer plot of the respective area over fluorescence induction. Note the difference in the slope of the two linear traces.

are connected via the peripheral Chl *a/b* LHC II and form a 'statistical' pigment bed cluster in which the participating photochemical reaction centers compete for the available excitation. In the partially restored curves, a fraction of open centers receive excitation from the collective antenna of the cluster, resulting in the higher rate of photon trapping. Fig. 6A (inset) shows the initial slopes of the α component from which the rate of photon trapping by open PS II_α centers was estimated. The rate of photon trapping by open PS II centers is plotted versus the fraction of open PS II centers in Fig. 7. Fig. 7 (PS II_α) shows that, under our actinic light conditions and when all PS II_α centers are open (100%), the rate of photon trapping is about 12 s^{-1}. Proportional with the closure of PS II_α centers, the rate of photon trapping increases so that when the fraction of open PS II_α centers is about 25%, the rate of photon trapping has tripled to about 36 s^{-1}. This observation is in agreement with the non-linear nature of the photoreduction of PS II_α, as manifested in the sigmoidal fluorescence induction curve and in the slope K_α of the semilogarithmic plot of the α component, which increases progressively with the photoreduction phenomenon [2,6]. Such a kinetically complex phenomenon was not observed for PS II_β. In chloroplasts poisoned with DCMU the rate of light absorption K_β (about 6 s^{-1}) was independent of the fraction of open PS II_β centers, shown in Fig. 7 (solid circles). A similar evaluation was undertaken for PS II_β directly from the restoration of the initial fluorescence rise from F_0 to F_{p1} in ferricyanide-added and NH_2OH-treated chloroplasts. Fig. 6B shows two fluorescence induction traces obtained with FeCN-treated chloroplasts that were either dark-adapted (D) or preilluminated for 2 s and allowed to restore for 0.5 s before the registration of the fluorescence induction trace. Both traces appear exponential in nature; however, the partially restored trace appeared slightly faster, and this was verified from the comparison of the respective semilogarithmic plots, shown in Fig. 6B (inset). Fig. 7 summarizes the results obtained in the absence of DCMU by showing the dependence of K_β (rate of light absorption by PS II_β) on the fraction of open PS II_β centers. Working both with FeCN-incubated (triangles) and NH_2OH-treated chloroplasts (squares),

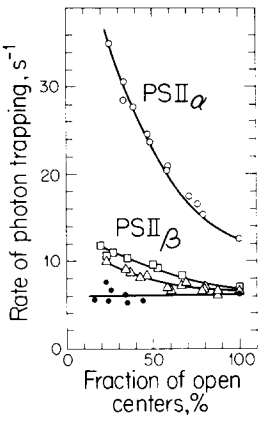

Fig. 7. The rate of photon trapping by open PS II_α and PS II_β reaction centers plotted as a function of the fraction of open centers. The data were derived from experiments similar to those shown in Fig. 6 with isolated spinach thylakoid membranes in the presence of DCMU (○, ●), in the presence of 2 mM potassium ferricyanide (△) and with hydroxylamine-treated chloroplasts in the absence of any further additions (□). Note the strong dependence of K_α (rate of photon trapping by open PS II_α centers) on the fraction of open PS II_α centers (○) and the independence of K_β, the rate of photon trapping by open PS II_β centers, on the fraction of open PS II_β centers in the presence of DCMU (●). Also note the weak dependence of K_β on the fraction of open PS II_β centers when determined in the presence of 2 mM ferricyanide (△) and with NH_2OH-treated chloroplasts (□).

we observed that K_β changed from a value of 6.5–7.0 s^{-1} in dark-adapted chloroplasts to about 11 s^{-1} when the fraction of open PS II_β centers was reduced to about 20% of the total PS II_β present. Such a result clearly deviates from the corresponding one observed in the presence of DCMU (Fig. 7, solid circles), and it may suggest that a partial connectivity does exist among PS II_β units. The 1.5–1.7-times increase in the value of K_β, however, does not in any way match the 3- to 4-fold increase in K_α observed under comparable conditions. The discrepancy of the results in the presence of DCMU may point to a DCMU interference with interunit excitation transfer, in agreement with earlier studies [13,14].

Discussion

The results presented in this work suggest that PS II_β activity is detected both in the absence of herbicides and in leaves under in vivo conditions.

In the absence of herbicides, PS II_β activity is manifested in the initial fluorescence yield rise from F_0 to F_{p1} which is kinetically complete before the full plastoquinone photoreduction. This conclusion is supported by three independent lines of evidence, summarized in Table I: (a) the rate of the initial fluorescence rise from F_0 to F_{p1} is identical to that of the β-component (K_β) detected in DCMU-poisoned chloroplasts, (b) the amplitude of the initial fluorescence rise from F_0 to F_{p1} is identical to that of the β-component ($F_{v\beta}$), and (c) the amount of Q photoreduced during the F_0-to-F_{p1} transition (Q_β/Chl, 1 : 1520) is about 26% of the total Q present (total Q/Chl, 1 : 390), in agreement with the 25% PS II_β content estimated from the biphasic kinetic analysis of DCMU-poisoned chloroplasts. The completion of the F_0 to F_{p1} transition before the full plastoquinone photoreduction would then be explained by the assumption that PS II_β, in addition to lacking the major portion of the peripheral Chl *a/b* LHC II, lacks the intermediate plastoquinone pool from the thylakoid membrane in which PS II_β resides. This hypothesis is supported by several observations: (1) Upon addition of oxidized quinone molecules to isolated chloroplasts the fluorescence rise from F_0 to F_{p1} is selectively quenched (Fig. 5, also Ref. 12). Other artificial electron acceptors such as ferricyanide, dichlorophenol-indophenol, methyl viologen and ferredoxin-$NADP^+$ are unable to effect this activation of PS II_β (Fig. 1, also Ref. 12). (2) Thielen and Van Gorkom [18] have reported the absence of the two-electron gate from PS II_β, in agreement with the proposed absence of plastoquinone from the thylakoid membrane in which PS II_β is localized. (3) It is known that far-red illumination ($\lambda > 690$ nm) readily restores the F_{p1} to F_{max} portion of the fluorescence induction curve, presumably because of the oxidation of the plastoquinone pool by PS I [19]. We observed that far-red illumination does not restore the F_0-to-F_{p1} transition efficiently. In far-red illuminated chloroplasts, we observed a fully oxidized plastoquinone pool; however, the initial amplitude of the fluorescence yield was near the F_{p1} level with the major portion of the transition from F_0 to F_{p1} missing (not shown). (4) Upon mechanical fractionation of spinach chloroplasts, PS II_β is isolated with the light ($100\,000 \times g$) membrane fraction [3]. It was observed that addition of herbicides to the latter did not alter the slow exponential fluorescence kinetics. This was in sharp contrast with the PS II_α-enriched fractions in which DCMU addition accelerated the fluorescence rise curve significantly. (5) A short saturating flash fully sufficed to eliminate the F_0 to F_{p1} transition in isolated chloroplasts [9].

In mature spinach and other higher plant chloroplasts, PS II_β accounts for a small fraction of the total PS II present (see Table I). Thus, the absence of electron transfer from PS II_β to PQ will not have a significantly adverse effect on PS II electron-transport capacity. It is worth pointing out, however, that PS II_β is not always synonymous with the absence of electron transfer to PQ. Exceptions to this rule may include photosynthetic mutant and developing chloroplasts which have lowered Chl *a/b* LHC II and enhanced PS II_β content. Such a notable example is the Chl *b*-lacking chlorina f_2 mutant of barley in which PS II_α is entirely absent. In this case, a substantial portion of PS II_β in the chloroplast (up to 80%) is functionally connected with the plastoquinone pool (Ghirardi, M.L. and Melis, A., unpublished data). Moreover, it is possible that PS II_α centers with damaged herbicide-binding protein, and therefore unable to transfer electrons to PQ in the grana partition region, would also contribute to the FeCN insensitive initial fluorescence rise from F_0 to F_{p1} [20]. In this case, however, the F_0 to F_{p1} transition would appear kinetically biphasic, reflecting the presence of both PS II_α and PS II_β in the measurement. This observation will explain the results of Forbush and Kok [9] who qualitatively discerned two kinetic components (F_0 to F_p, and F_p to F_{p1}) in the initial fluorescence rise. Furthermore, it is conceivable that during illumination of chloroplasts and because of the ensuing movement of PQ in the thylakoid membrane PS II_β becomes 'activated', as proposed by Joliot [11].

The results presented in this work support the notion that PS II_β differs from PS II_α because it lacks a substantial portion of the peripheral Chl *a/b* LHC II [1–6], and also because of the absence of the intermediate plastoquinone pool from the thylakoid membrane in which it is localized. There is evidence in the literature supporting the presence of all other functional system II components

TABLE II

QUANTITATION OF SYSTEM II COMPONENTS IN SPINACH CHLOROPLASTS

A variety of experimental procedures from different laboratories measured the concentration of the following integral PS II components: Mn, Z (secondary donor of PS II), cytochrome *b*-559, P-680 (reaction center of PS II), Q (stable primary electron acceptor of PS II) and herbicide binding sites. The number in parentheses indicate the mol amount of the respective component per mol PS II complex.

Component quantitated		Chl/component ratio	Reference
Mn	(4)	≈ 400	25, 26
Z [a]	(1)	390 ± 30	27
Cyt *b*-559	(2)	≈ 350 [b]	4, 28
P-680	(1)	360 ± 40	27, 29, 30
Q	(1)	350 ± 50	this work, 6, 24
Herbicide binding sites	(1)	390 ± 20	31, 32

[a] Measured from the light-induced signal II.

[b] Estimated from the concentration of cyt *b*-559 in resolved membrane preparations from the grana partition region.

in PS II_β. Table II summarizes the concentration of functional PS II components in spinach chloroplasts, on a total Chl($a + b$) basis. It is observed that the ratio of Chl/(4 Mn, 2 Cyt *b*-559, 1 Z, 1 P-680, 1 Q, 1 herbicide-binding protein) is consistently found in the range of 350–400, suggesting that all afore-mentioned System II functional components are present both in PS II_α and in PS II_β. There is further evidence in the literature that under repetitive excitation of low frequency PS II_β is capable of evolving oxygen [21,22]. Assuming that PS II_β accounts for about 25% of the total PS II present in spinach chloroplasts (Table I), the average ratio Chl/PS II, 390 : 1 (Table I and II; also Refs. 1–6 and 23–32) would be resolved further into Chl/PS II_α/PS II_β as 390 : 0.75 : 0.25. Thus, on a total Chl basis, the concentration of PS II_α and PS II_β in spinach chloroplasts is given by Chl/PS II_α as 520 : 1 and Chl/PS II_β as 1560 : 1, in agreement with Refs. 12 and 23.

Our analysis provides the basis for predicting oxygen flash yield and proton flash yield values in isolated spinach chloroplasts, depending on the artificial electron acceptor system used. In the presence of dichlorophenol-indophenol and also in the presence of relatively high concentrations of potassium ferricyanide (> 500 μM), such that ferricyanide reduction is not limited by PS I turnover [12], we would predict a maximum PS II yield of 1 e^- equivalent per flash per about 520 Chl molecules, reflecting the activity of PS II_α. However, when DMQ or TMQ is used in combination with ferricyanide (provided that care is exercised to avoid several artifacts, including static quenching of excitation energy by added quinones [33], inhibition of H_2O-splitting activity by potassium ferricyanide [34], and a possibly poor equilibration between lipophilic quinones and hydrophilic ferricyanide [12]), we would predict higher oxygen and proton flash yield values approaching 1 e^- equivalent per flash per about 390 Chl molecules, reflecting the activity of both PS II_α and PS II_β. Indeed, in a recent study with spinach chloroplasts from freshly harvested leaves, we demonstrated that PS II electron-transport capacity is approaching 1 e^- equivalent per about 390 Chl molecules when experimental conditions are carefully checked [12]. In contrast, also in a recent study with chloroplasts from spinach purchased from the commercial market (spinach grown in different geographical regions of the U.S.A. and Canada), Whitmarsh and Ort used DMQ and ferricyanide as the artificial electron acceptors and reported a PS II yield of 1 H^+ per flash per about 630 Chl molecules [35]. Our interpretation of their result is that a Chl/PS II ratio of 630 : 1 probably reflects the concentration of active PS II centers in their samples and not the true PS II concentration in spinach chloroplasts. Since Whitmarsh and Ort used DMQ and ferricyanide concentrations sufficient to enable electron flow through both PS II_α and PS II_β, it is not immediately clear why their proton flash yield values are as low as 1 equivalent per flash per about 630 Chl molecules instead of per about 390 Chl molecules. Efforts are currently under way to explain this discrepancy.

The results from this work, along with the findings of a recent chloroplast developmental study from this laboratory [24] point to a developmental relationship between PS II_α and PS II_β. As a working hypothesis, we propose that PS II_α assembly and grana formation occurs in a two-step process, the first of which corresponds to the formation of PS II_β in stroma-exposed thylakoid membrane segments. The second developmental

step occurs upon the addition of plastoquinone, of the peripheral Chl *a/b* LHC II and the ensuing incorporation of this membrane segment into a granum partition region. This working model is substantially different from that proposed earlier by Melis and Akoyunoglou [36], and it would require further rigorous examination. If valid, it may provide an answer both with respect to the physiological significance of PS II heterogeneity in higher plant chloroplasts and with respect to the question of PS II_α assembly and grana formation.

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References

1 Melis, A. and Homann, P.H. (1976) Photochem. Photobiol. 23, 343–350
2 Melis, A. and Duysens, L.N.M. (1979) Photochem. Photobiol. 29, 373–382
3 Anderson, J.M. and Melis, A. (1983) Proc. Natl. Acad. Sci. USA 80, 745–749
4 Lam, E., Baltimore, B., Ortiz, W., Melis, A. and Malkin, R. (1983) Biochim. Biophys. Acta 724, 201–211
5 Thielen, A.P.G.M. and Van Gorkom, H.J. (1981) Biochim. Biophys. Acta 635, 111–120
6 Melis, A. and Anderson, J.M. (1983) Biochim. Biophys. Acta 724, 473–484
7 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
8 Melis, A. and Hart, R.W. (1980) Carnegie Yearbook 79, 170–172
9 Forbush, B. and Kok, B. (1968) Biochim. Biophys. Acta 162, 243–253
10 Thielen, A.P.G.M., Van Gorkom, H.J. and Rijgersberg, C.P. (1981) Biochim. Biophys. Acta 635, 121–131
11 Joliot, P. (1965) Biochim. Biophys. Acta 102, 116–148
12 McCauley, S.W., Taylor, S.E., Dennenberg, R.J. and Melis, A. (1984) Biochim. Biophys. Acta 765, 186–195
13 Ramanujam, P. and Bose, S. (1983) Photochem. Photobiol. 37, 77–80
14 Horvath, G., Droppa, M. and Melis, A. (1984) Photobiochem. Photobiophys. 7, 249–256
15 Doschek, W.W. and Kok, B. (1972) Biophys. J. 12, 832–838
16 Joliot, P., Bennoun, P. and Joliot, A. (1973) Biochim. Biophys. Acta 305, 317–328
17 Melis, A. (1975) Dissertation. The Florida State University, Tallahassee, Fl.
18 Thielen, A.P.G.M. and Van Gorkom, H.J. (1981) in Photosynthesis, Proceedings of the 5th International Congress (Akoyunoglou, G., ed.), Vol. II, pp. 57–64, Balaban International Science Services, Philadelphia, PA
19 Malkin, S. and Kok, B. (1966) Biochim. Biophys. Acta 126, 413–432
20 Vermaas, W.F.J., Arntzen, C.J. Gu, L.U., Yu, C.A. (1983) Biochim. Biophys. Acta 723, 266–275
21 Jursinic, P. (1979) Arch. Biochem. Biophys. 196, 484–492
22 Theg, S.M. and Homann, P.H. (1981) in Photosynthesis, Proceedings of the 5th International Congress (Akoyunoglou, G., ed.), Vol. I, pp. 309–318. Balaban International Science Services, Philadelphia, PA
23 Ghirardi, M.L. and Melis, A. (1984) Plant Physiol. 74, 993–998
24 Melis, A. (1984) J. Cell Biochem. 24, 271–285
25 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (9181) FEBS Lett. 134, 231–234
26 Yocum, C.F., Yerkes, C.T., Blankenship, R.E., Sharp, R.R. and Babcock, G.T. (1981) Proc. Natl. Acad. Sci. USA 78, 7507–7511
27 Babcock, G.T., Ghanotakis, D.F., Ke, B. and Diner, B.A. (1983) Biochim. Biophys. Acta 723, 276–286
28 Murata, N., Miyao, M., Omata, T., Matsunami, H. and Kuwabara, T. (1984) Biochim. Biophys. Acta 765, 363–369
29 Van Best, J.A. and Mathis, P. (1978) Biochim. Biophys. Acta 503, 178–188
30 Eckert, H.-J., Renger, G. and Witt, H.T. (1984) FEBS Lett. 167, 316–320
31 Vermaas, W.F.J. and Artnzen, C.J. (1983) Biochim. Biophys. Acta 725, 483–491
32 Jursinic, P. and Stemler, A. (1983) Plant Physiol. 73, 703–708
33 Kitajima, M. and Butler, W.L. (1975) Biochim. Biophys. Acta 376, 105–115
34 Dreschler, A. and Neumann, J. (1982) Plant Physiol. 70, 840–843
35 Whitmarsh, J. and Ort, D.R. (1984) Arch. Biochem. Biophys. 231, 378–389
36 Melis, A. and Akoyunoglou, G. (1977) Plant Physiol. 59, 1156–1160