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Evidence for slow turnover in a fraction of Photosystem II complexes in thylakoid membranes

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We used two different techniques to measure the recovery time of Photosystem II following the transfer of a single electron from P-680 to Q_A in thylakoid membranes isolated from spinach. Electron transfer in Photosystem II reaction centers was probed first by spectroscopic measurements of the electrochromic shift at 518 nm due to charge separation within the reaction centers. Using two short actinic flashes separated by a variable time interval we determined the time required after the first flash for the electrochromic shift at 518 nm to recover to the full extent on the second flash. In the second technique the redox state of Q_A at variable times after a saturating flash was monitored by measurement of the fluorescence induction in the absence of an inhibitor and in the presence of ferricyanide. The objective was to determine the time required after the actinic flash for the fluorescence induction to recover to the value observed after a 60 s dark period. Measurements were done under conditions in which (1) the electron donor for Photosystem II was water and the acceptor was the endogenous plastoquinone pool, and (2) Q_{400}, the Fe^{2+} near Q_A, remained reduced and therefore was not a participant in the flash-induced electron-transfer reactions. The electrochromic shift at 518 nm and the fluorescence induction revealed a prominent biphasic recovery time for Photosystem II reaction centers. The majority of the Photosystem II reaction centers recovered in less than 50 ms. However, approx. one-third of the Photosystem II reaction centers required a half-time of 2–3 s to recover. Our interpretation of these data is that Photosystem II reaction centers consist of at least two distinct populations. One population, typically 68% of the total amount of Photosystem II as determined by the electrochromic shift, has a steady-state turnover rate for the electron-transfer reaction from water to the plastoquinone pool of approx. 250 e^-/s, sufficiently rapid to account for measured rates of steady-state electron transport. The other population, typically 32%, has a turnover rate of approx. 0.2 e^-/s. Since this turnover rate is over 1000-times slower than normally active Photosystem II complexes, we conclude that the slowly turning over Photosystem II complexes are inconsequential in contributing to energy transduction.

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Abbreviations: PS I, Photosystem I; PS II, Photosystem II; ΔA_{518}, flash-induced absorbance change measured at 518 nm; Chl, chlorophyll; DCMU, 3[3,4-dichlorophenyl]-1,1-dimethylurea; F_v, variable fluorescence intensity; F_i, initial fluorescence intensity; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic aicd; Mes, 4-morpholineethanesulophonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; P-680, primary electron donor of Photosystem II; P-700, primary electron donor of Photosystem I; Q_A, primary acceptor quinone for Photosystem II; Q_B, secondary acceptor quinone for Photosystem II; Q_{400}, Photosystem II acceptor with redox potential of 400 mV.

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The slowly turning over Photosystem II complexes are able to transfer an electron from P-680 to Q_A rapidly, but the reoxidation of Q_A^- is slow ($t_{1/2}$ = 2 s). The fluorescence induction measurements lead us to conclude that there is significant overlap between the slowly turning over fraction of Photosystem II complexes and PS II_β reaction centers. One corollary of this conclusion is that electron transfer from P-680 to Q_A in PS II_β reaction centers results in charge separation across the membrane and gives rise to an electrochromic shift.

Introduction

In normally functioning Photosystem II complexes electrons from H_2O reduce bound plastoquinone resulting in the release of plastoquinol into the hydrophobic region of the thylakoid membrane [1–3]. The two-electron reduction of plastoquinone requires two sequential photochemical reactions and involves two bound quinones, Q_A and Q_B, operating in series. In reaction centers in which Q_A and Q_B are initially oxidized the first light reaction drives an electron from P-680 to pheophytin, which in turn reduces Q_A creating a relatively stable charge separation across the membrane. Subsequently, the electron on Q_A equilibrates between Q_A and Q_B with the state of electron sharing favoring Q_B. In the second light reaction an electron is transferred over the same path to Q_B^- and together with two protons results in the reduction of Q_B to QH_2. Plastoquinol then debinds from Photosystem II leaving the Q_B-site available for oxidized plastoquinone. The steady-state turn-over rate of normally functioning Photosystem II complexes is typically 200–300 e^-/s indicating that each Photosystem II complex must on average turn over every few ms.

While this view of Photosystem II is sufficient to account for a vast number of observations there are various elements of Photosystem II that exhibit heterogenous behavior not easily accounted for by the above model [4–12]. These elements include Q_A, Q_B, Q_{400} (the Fe^{2+} located near Q_A), cytochrome *b*-559, and the antenna system serving the reaction centers (reviewed in Refs. 1, 2 and 13). The observed heterogeneity has prompted diverse models of Photosystem II, several of which include a fraction of complexes that appear to be inactive when compared to the normally functioning complexes [5–7,9–12]. Lavergne [6,7] investigated the Q_B-site of Photosystem II by fluorescence measurements and concluded that Photosystem II is divided between Q_B and non-Q_B type reaction centers. He proposed that a fraction of the reaction centers do not transfer an electron from water to the plastoquinone pool at physiological rates. In an earlier study Thielen and Van Gorkom [5] measured fluorescence properties in PS II_α and PS II_β centers and concluded that the reduction of Q_B in PS II_β did not occur by the two electron process. Furthermore, Van Gorkom suggested that electron transfer from P-680 to Q_A in PS II_β may not be electrogenic [2]. The notion that PS II_β centers may be impaired in electron transfer from Q_A to Q_B has recently been supported by Melis [9] who measured fluorescence induction in thylakoid membranes in the absence of inhibitors and the presence of ferricyanide. He concluded that PS II_β centers do not transfer electrons rapidly to the plastoquinone pool. Further evidence for functionally different reaction centers is provided by recent results from Graan and Ort [10] who measured the concentration of Photosystem II complexes by the flash-induced yield of protons using different electron acceptors and by the number of Q_B-sites capable of binding the inhibitor terbutryn. They found that certain halogenated benzoquinones increased the number of Photosystem II complexes detected by flash-induced turnover and concluded that approx. 40% of the Photosystem II complexes do not transfer electrons to plastoquinone at physiological rates in thylakoid membranes.

In this work our interest centers on the functional heterogeneity of photosystem II in thylakoid membranes. We have investigated this problem by probing the turnover time of photosystem II in which H_2O is the electron donor and endogenous plastoquinone the electron acceptor. The objective was to measure the reoxidation time of Q_A^- subsequent to a saturating flash by monitoring the electrochromic shift at 518 nm and the fluorescence induction. Our data indicate that in 32% of

Photosystem II complexes in thylakoid membranes the reoxidation half-time of Q_A^- is 2 s, 1000-times slower than normally active Photosystem II complexes. Below we discuss these observations in the context of previous explanations of Photosystem II heterogeneity and examine the extent of the overlap between the inactive Photosystem II complexes described here and PS II_β centers. We identify at least a fraction of the inactive Photosystem II complexes with PS II_β, which offers a resolution of the controversy surrounding the amount of Photosystem II present in thylakoid membranes [5–7,9–12]. Based on the electrochromic shift we calculate that the ratio of rapidly turning over Photosystem II to PS I is 1.1 ± 0.1.

Preliminary accounts of this work have been presented [11,12].

Materials and Methods

All experiments were carried out using thylakoid membranes isolated from market spinach (*Spinacia oleracea*) as described elsewhere [14]. The chlorophyll concentration was determined in acetone/water (4 : 1) using the extinction coefficients for chlorophyll *a* and chlorophyll *b* at 664 nm and 647 nm determined by Ziegler and Egle [15]. The thylakoid membranes were stored on ice in a medium containing 0.2 M sorbitol, 5 mM Hepes-KOH (pH 7.5), 2 mM $MgCl_2$, 0.5 mg per ml bovine serum albumin (essentially fatty acid free) and used for experiments from 1 to 5 h after isolation.

For spectrophotometric measurements at pH 8.0 the thylakoid membranes were suspended in a reaction medium containing 0.1 M sorbitol, 30 mM Tricine/KOH (pH 8.0), 20 mM KCl, 2 mM $MgCl_2$, 0.1 mM methyl viologen. Other additions are indicated in the legends. Gramicidin D or valinomycin (Sigma, St. Louis, MO), when present, were added from stock solutions in ethanol so that the volume of ethanol in the reaction mixture never exceeded 0.1%. For spectrophotometric measurements made at lower pH values the Tricine was replaced by either Hepes or Mes as indicated in the legends. The flash-induced absorbance changes were signal averaged to improve the signal-to-noise ratio. Samples were changed so that the thylakoids received less than 100 actinic flashes. The pathlength of the measuring beam was 1 cm. All measurements were done using samples thermostatted at 18° C.

Absorbance changes were measured using a laboratory-built spectrophotometer. The measuring beam was produced by a 55 W tungsten-halogen lamp and was blocked by an electronic shutter to prevent photochemical reactions prior to recording traces. The half-bandwidth of the measuring beam was 4 nm. The shutter was opened between 50 and 200 ms prior to the actinic flash. Actinic flashes were produced by a xenon flash lamp (FX-193 Electric Optics) filtered by a red blocking filter (Corning Glass Works, Corning, NY, CS-2-58) directed through one of three light guides at right angles to the measuring beam. The half-peak width of the actinic flash was 6 μs, which produced less than 8% double hits as compared to a 0.5 μs duration laser flash. For all the measurements shown here the actinic flash was saturating. The photomultiplier tube (EMI 9634 QR, Thorn-EMI, Plainview, NY) was protected from the actinic flash by a blocking filter (CS-4-96, Corning Glass Works, Corning, NY) and a broad band interference filter (DT-Gruen, Balzers, Rolyn Optics, Arcadia, CA). The absorbance changes were recorded using a Biomation Waveform Recorder (Model 805, Biomation Corporation, Cupertino, CA) and stored in a Nicolet Signal Averager (Model 1174, Nicolet Instrument Corp., Madison, WI).

The oxidation state of P-700 in the presence of the far-red background light was measured using a modified DW2*c* spectrophotometer (SLM/Aminco, Urbana, IL) as described elsewhere [14].

Far-red background light, when used, was produced by a 250 W tungsten-halogen lamp filtered by a combination of a 730 nm interference filter (10 nm half-bandwidth, Corion), a red blocking filter (CS 2-58, Corning) and a heat absorbing filter (CS I-57, Corning). The intensity of the far-red light was measured using a YSI Model 65A Radiometer (Yellow Springs, OH).

Fluorescence measurements were done using a laboratory-built fluorimeter and recorded using a Nicolet Signal Averager (Model 1174, Nicolet Instrument Corp., Madison, WI). Actinic light was provided by a 250 W tungsten-halogen lamp

filtered by Corning CS 4-96 and CS 3-96 blocking filters. Fluorescence intensity was detected by a photomultiplier tube (EMI 9558 QA, Thorn-EMI Corp., Holliston, NY) protected from exciting light by a Corning CS 2-58 blocking filter and a Schott 630 blocking filter.

Results

ΔA_{518}

In the reaction centers of thylakoids a short actinic flash drives an electron across the membrane creating an electric field which causes a shift in the absorption spectrum of carotenoids and chlorophyll that can be monitored by a rapid absorbance increase at 518 nm [16,17]. In Photosystem II the transmembrane charge transfer is accounted for by electron transfer from P-680 to pheophytin to Q_A [18]. In Photosystem I the transmembrane charge transfer is accounted for by electron transfer from P-700 to chlorophyll *a* [19,20]. In the experiments described in this section we measured the extent of the rapid absorbance increase at 518 nm induced by a short flash in order to determine the number of reaction centers capable of transferring an electron across the membrane to a stable acceptor. The objective was to measure the turnover activity of the reaction centers by determining the time required subsequent to a saturating flash for 100% recovery of the signal [21,22]. The principal experiment was to illuminate thylakoids with two actinic flashes separated by a variable time and to compare the extent of the rapid absorbance increase at 518 nm due to the second flash to that due to the first flash. A typical example of the gramicidin-sensitive absorbance change at 518 nm induced by two flashes separated by 200 ms for the electron-transfer reaction from H_2O to methyl viologen is shown in Fig. 1. The extent of the gramicidin sensitive ΔA_{518} induced by the second flash was 0.80 ± 0.015 of that induced by the first flash. This result indicates that 200 ms after a saturating flash approx. 20% of the reaction centers observed in the first flash were unable to create a stable charge separation across the membrane. Prior to the first flash the thylakoid suspension was dark adapted for 30 s.

The gramicidin-sensitive ΔA_{518}, defined as the

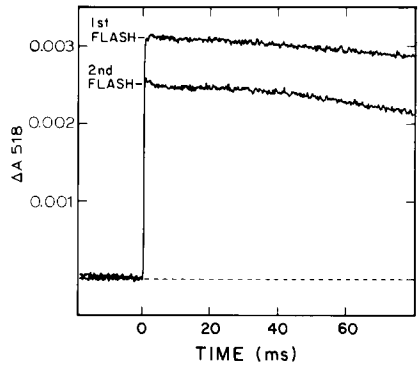

Fig. 1. The gramicidin-sensitive absorbance change observed at 518 nm in spinach thylakoids due to two different flashes. The figure shows two kinetic traces superimposed in order to compare the extent of the rapid absorbance increase. The trace labeled first flash was measured after a 30 s dark period. The second flash was given 200 ms after the first flash. Each trace is the absorbance change observed in the absence of gramicidin minus the absorbance change observed in the presence of gramicidin. Each trace is the average of 80 runs measured at an instrument response time of 100 μs. The thylakoid membranes were suspended at a chlorophyll concentration of 30 μM in a reaction medium that contained 0.1 M sorbitol, 30 mM Tricine/KOH (pH 8.0), 20 mM KCl, 2 mM $MgCl_2$, 0.1 mM methyl viologen, and 10 μg/ml gramicidin when present. See text for further details.

flash-induced ΔA_{518} measured in the absence minus that measured in the presence of gramicidin, was measured in order to monitor the electrochromic shift without interference from other light-induced absorbance changes [21,23]. The gramicidin-sensitive fraction of the signal is typically 85–95% of the absorbance change observed in the absence of gramicidin. In this work we assume that the gramicidin-sensitive flash induced ΔA_{518} is linearly proportional to the electric field across the membrane [17], a reasonable assumption when the electric field is created by one or two flashes given to dark-adapted thylakoids.

The time required for full recovery of the reaction centers subsequent to a flash was determined by changing the time between the two flashes (Fig. 2). The data reveal a prominent biphasic recovery; 77% of the ΔA_{518} signal recovers within 100 ms (time-course not resolved), while the remaining 23% of the ΔA_{518} signal recovers with a half-time of 1.8 s. On average we found 80% of the A_{518} recovered faster than 50 ms after a flash. The total flash-induced absorbance change at 518 nm exhibits essentially the same characteristics as shown

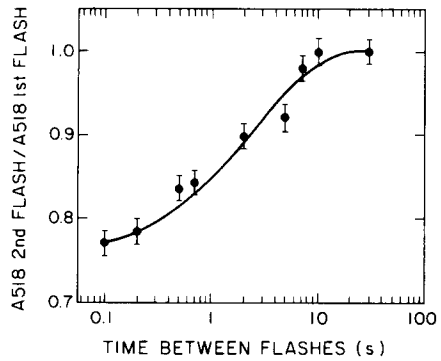

Fig. 2. Recovery of the gramicidin-sensitive, flash-induced absorbance change at 518 nm in thylakoids as a function of the time between the first and second flash. The recovery of A_{518} is defined as the extent of the rapid absorbance increase due to the second flash divided by the extent of the rapid absorbance increase due to the first flash. An example is shown in Fig. 1. Experimental conditions and the reaction mixture were the same as described in the legend of Fig. 1. The error bars represent the estimated standard deviation.

here for the gramicidin-sensitive fraction [11,12]. Table I shows that the average amount of the slow-recovering ΔA_{518} was $20 \pm 3\%$ with an average half-time of 2.3 ± 0.5 s, whereas $80 \pm 3\%$ of the ΔA_{518} recovered faster than 200 ms. The possibility that the transmembrane electric field created on the first flash reduced the amount of stable charge transfer on the second flash is unlikely, since the decay half-time of the transmembrane electric field was 400–600 ms (data not shown) and the recovery time of the slowly recovering ΔA_{518} was 2 s (Fig. 2).

In order to determine the contribution of Photosystem II to the gramicidin-sensitive ΔA_{518} in the reaction from H_2O to methyl viologen, thylakoids were illuminated with continuous far-red light to oxidize P-700 so that the observable ΔA_{518} was due solely to Photosystem II. We found that the slowly turning-over reaction centers saturated at far-red light intensities greater than 50 W/m^2 and were therefore unable to turnover in a short flash (see Ref. 11 for a detailed discussion). Therefore, in the presence of far-red background light the ΔA_{518} is due to the rapidly turning over fraction of Photosystem II. The rapidly turning over Photosystem II complexes accounted for $42 \pm 1\%$ of the control ΔA_{518} induced by a short flash in the electron reaction from H_2O to methyl viologen (Table I).

In Fig. 3 the pH dependence of the recovery of the gramicidin-sensitive ΔA_{518} using a single thylakoid preparation is shown. We found that the extent of the recovery, measured between 50 and 200 ms after a flash, was nearly pH independent. We compared the recovery of the A_{518} at pH 6.5

TABLE I

SUMMARY OF THE RECOVERY OF ΔA_{518} AT pH 8.0 FOR DIFFERENT THYLAKOID PREPARATIONS

The extent of the ΔA_{518} (2nd flash) was determined between 100 and 200 ms after a flash. The extent of ΔA_{518} (1st flash) was determined 30 s after a flash. Experimental conditions were identical to those described in Figs. 1 and 2, except in some experiments the total ΔA_{518} was measured rather than the gramicidin-sensitive fraction. There was no observable difference between the two cases. The uncertainty shown is the standard deviation. The intensity of the far-red light was 45–100 W/m^2.

	Number of thylakoid preparations	Range	Average
$\frac{\Delta A_{518}\ (2nd\ flash)}{\Delta A_{518}\ (1st\ flash)}$	16	74 –85%	80 ±3%
ΔA_{518} (slow recovery)	16	15 –26%	20 ±3%
$t_{1/2}$ (slow recovery)	5	1.8– 3.0 s	2.3±0.5 s
ΔA_{518} + far-red light	5	40 –44%	42 ±1%

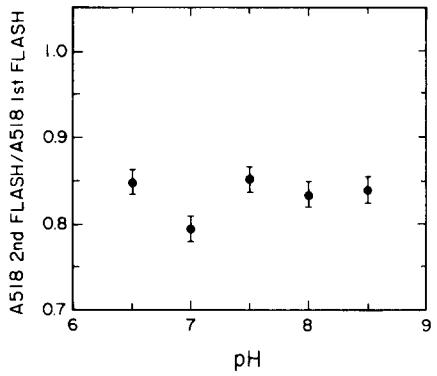

Fig. 3. pH dependence of the recovery of the gramicidin-sensitive, flash-induced absorbance change at 518 nm in thylakoids. The recovery was determined as described in fig. 2, except the second flash was given between 50 and 200 ms after the first flash. The reaction conditions were the same as described in Fig. 1 except the pH was titrated to the value indicated. At pH 6.5 the buffer was Mes, and at pH 7.0 and 7.5 the buffer was Hepes rather than Tricine. The thylakoids were incubated in the reaction medium for at least 3 min prior to the measurements.

and 8.0 for five different thylakoid preparations. At pH 8.0 the average recovery was 77 ∓ 5% and at pH 6.5 the recovery was ∓6%. At pH 6.5 the half-time for the recovery of the slowly turning over fraction was 3 s [11].

Attempts to determine the effect of 2,6-dichloro-*p*-benzoquinone on the oxidation rate of Q_A^- measured by the ΔA_{518} due to the second flash were unsuccessful. This was likely due to partial oxidation of P-700 by 2,6-dichloro-*p*-benzoquinone, which resulted in a variable extent of ΔA_{518}.

Fluorescence induction

Fluorescence induction measurements in thylakoids consists of determining the chlorophyll fluorescence yield as a function of time during continuous illumination. The fluorescence yield is a sensitive function of the redox state of Q_A, and is used here to determine the oxidation kinetics of Q_A^- [24,25]. Early studies of fluorescence induction using thylakoids in the presence of ferricyanide and the absence of a Q_B site inhibitor revealed a small induction phase labelled F_{pl}, where F_{pl} is the fluorescence at plateau level [24]. We determined the effect of pretreating thylakoids with a single turnover flash upon the F_{pl} fluorescence induction kinetics. The objective was to

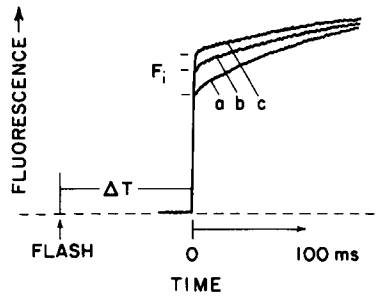

Fig. 4. Fluorescence induction in thylakoids measured at various times after a short actinic flash. ΔT is the dark time between the short flash and the onset of fluorescence induction driven by continuous light. F_i is the initial fluorescence intensity observed immediately upon illumination by the continuous light. F_v is the fluorescence itnensity measured 100 ms after the onset of the continuous flash minus F_i. The experimental conditions were identical to those described in Fig. 1, except $MgCl_2$ was present at 5 mM and 0.25 mM ferricyanide was present. (a) Fluorescence induction measured after a 60 s dark period. (b) Fluorescence induction measured 1 s after a short actinic flash. (c) Fluorescence induction measured 200 ms after a short actinic flash.

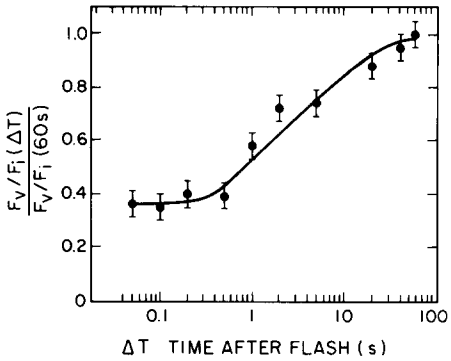

Fig. 5. Recovery of F_v/F_i plotted as a function of the time (ΔT) after a short actinic flash. F_v/F_i was determined as in Fig. 5. The y-axis is F_v/F_i measured at the time ΔT after the short flash divided by F_v/F_i measured after a 60 s dark time. See Fig. 5 and the text for further details. Experimental conditions were identical to those described in Fig. 5.

measure the recovery time of the reaction centers giving rise to the F_{pl} fluorescence induction subsequent to an actinic flash. The experiment was similar to that done by Owens and Joliot (presented in Ref. 24) and consisted of measuring the fluorescence induction kinetics for thylakoids in the presence of ferricyanide and in the absence of an inhibitor (Fig. 4). In Fig. 5 the recovery of the F_{pl} fluorescence plotted against the time between the actinic flash and the onset of the fluorescence induction kinetics is shown. The half-time required for full recovery was 3 s. Fluorescence induction measurements showed that Q_{400} remained reduced even in the presence of 0.25 mM ferricyanide because the sample was exposed to a flash every 60 s. The oxidation of Q_{400} in the presence of 0.25 mM ferricyanide is several minutes (data not shown).

Discussion

We interpret the slowly recovering phase of the flash-induced electrochromic shift as due to a fraction of reaction centers that require several seconds recovery time before they are capable of turning over a second time. The data reveal that on average, 20% of the reaction centers in thylakoid membranes require 2.3 s recovery time subsequent to a short flash before they are capable of turning over a second time. Firstly, we conclude that the slowly recovering phase of the ΔA_{518} is not due to Photosystem I reaction centers because the flu-

orescence induction due to Photosystem II measured in the presence of ferricyanide and in the absence of Q_B site inhibitor reveals a slowly turning-over fraction of Photosystem II reaction centers with a recovery half time of approx. 3 s. Secondly, we showed elsewhere that the ΔA_{518} measured under conditions where only Photosystem II could turn over exhibits a slowly turning-over component. These experiments were done using thylakoid membranes in the presence of ferricyanide and far-red background light that totally oxidized P-700, thereby preventing the flash-induced turnover of Photosystem I [11].

The amount of the slowly turning-over Photosystem II can be calculated from its contribution to the gramicidin-sensitive ΔA_{518}. Since on average $20 \pm 3\%$ of the gramicidin-sensitive ΔA_{518} is due to slowly recovering Photosystem II complexes and 42% is due to rapidly turning over Photosystem II complexes these measurements indicate that $32 \pm 3\%$ of the total Photosystem II exhibits slow recovery kinetics. According to these data, 38% of the gramicidin-sensitive ΔA_{518} is due to the Photosystem I. Therefore, the ratio of rapidly turning-over Photosystem II to total photosystem I based on the ΔA_{518} is 1.1 ± 0.1. This ratio is within the range of values we determined in an earlier study for the ratio of active Photosystem II to Photosystem I [14]. In the present analysis we have assumed that charge transfer in the slowly turning-over complexes spans the membrane. This assumption may not be correct in which case the contribution of slowly turning-over complexes may be larger than is indicated by the electrochromic shift.

The above interpretation of our data in terms of two distinct groups of Photosystem II is based on the assumption that in normally functioning complexes after a single flash the equilibrium of the electron between Q_A and Q_B favors $Q_AQ_B^-$ rather than $Q_A^-Q_B$, since $Q_A^-Q_B$ would effectively act as an inactive center during the second flash. In other words, we assumed that the equilibrium constant for the $Q_A^-Q_B/Q_AQ_B^-$ reaction is large [26–28]. Measurements by Robinson and Crofts [25], however, reveal a small apparent equilibrium constant at pH 8 (K_{app} near 6) that is strongly pH dependent, increasing to a value of 20 at pH 6.5. In contrast to the strong pH dependence determined by Robinson and Crofts for the apparent equilibrium constant for the $Q_A^-Q_B/Q_AQ_B^-$ reaction, we found that the amount of slowly turning-over complexes showed only a 10% change from pH 6.5 to 8.5. We interpret this result to indicate that the apparent equilibrium constant for active Photosystem II complexes at pH 8.0 of $Q_A^-Q_B/Q_AQ_B^-$ is 20 or larger, and as a consequence, the active Photosystem II centers in the state $Q_A^-Q_B$ do not significantly contribute to the slowly turning-over complexes we observe.

Another factor that must be considered in interpreting our results is the presence of Q_{400} [29–31], which in the Fe^{3+} state can act as an additional electron acceptor in photosystem II. In the experiments described here we selected experimental conditions that maintained Q_{400} in the reduced state so that it could not act as an electron acceptor.

Melis and Homann initially identified PS II_β by fluorescence induction measurements that they interpreted in terms of two populations of Photosystem II, PS II_α and PS II_β [32]. The PS II_β complexes have a smaller antenna size, and appear to be located predominantly in stroma-exposed membranes. Thielen and Van Gorkom [5] and Lavergne [6,7] have suggested that PS II_β centers are impaired at the Q_B-site and may not transfer electrons efficiently to the plastoquinone pool. Recently, Melis has also concluded that PS II_β are inactive based on fluorescence induction measurements done in the absence of inhibitor and the presence of ferricyanide [9]. We investigated the overlap between the inactive complexes and PS II_β by measuring the recovery time of the fluorescence induction after a saturating flash in the absence of inhibitor and the presence of ferricyanide. Our results show that the half-time for Q_A^- reoxidation is the same as that determined using the electrochromic shift. If we assume, as argued by Melis, that the fluorescence induction measured in the absence of inhibitor and the presence of ferricyanide is due to PS II_β centers [9], then our data show a strong overlap between inactive complexes and PS II_β.

Conclusion

Two different probes of Photosystem II turnover, the electrochromic shift and the fluores-

cence induction, each support the same conclusion – in thylakoid membranes Photosystem II exists as two distinct populations: defined here as active and inactive. The active complexes, comprising 68% of the total, are the normally functioning Photosystem II complexes that turnover at high rates (250 e^-/s) providing electrons for Photosystem I. The inactive complexes comprise the remaining 32% and turn over 1000-times slower than the active centers, 0.2 e^-/s. The turnover time of Photosystem II was determined by measuring the recovery time after a flash of the electrochromic shift and the fluorescence induction measured in the absence of an inhibitor and the presence of ferricyanide. The results show that the reoxidation half-time of Q_A^- after a flash is approx. 2 s in the inactive complexes. This conclusion is applicable for thylakoid membranes isolated from spinach under conditions where H_2O is the electron donor and endogenous plastoquinone is the electron acceptor for Photosystem II and either methyl viologen or ferricyanide is the electron acceptor for Photosystem I [11]. These experiments were done under conditions where Q_{400} remained reduced, so that complications due to an additional electron acceptor near Q_A do not apply to our results [33]. We have measured approximately the same fraction of inactive Photosystem II complexes in pea thylakoids (R. Chylla, W. Lee, and J. Whitmarsh, unpublished data).

At present we are unable to identify the electron acceptor for reoxidation of Q_A^- in the inactive complexes. Among the possible acceptors are plastoquinone at the Q_B-site turning over at a low rate, plastoquinone at the quinol reductase site of the cytochrome *b/f* complex, O_2, or a back reaction in Photosystem II to an unidentified acceptor. The last possibility is supported by the fact that the half-time for Q_A reoxidation we determined here for the inactive complexes is the same as the half-time determined for Q_A^- reoxidation under three different conditions where electron transfer to Q_B is impaired. (1) In the presence of DCMU, which prevents plastoquinone from binding to the Q_B site, the half-time for Q_A^- reoxidation is 2 s [34,35]. Renger and Wolff [34] showed that the reoxidation was altered by inhibition of water oxidation and concluded the Q_A^- reoxidation occurred by a back reaction. (2) In a separate study Renger and Weiss [36] exposed thylakoid membranes to trypsin which alters the Q_B site. Monitoring the reoxidation of Q_A^- at 320 nm they showed that the reoxidation half-time of Q_A^- was 2.3 s in trypsin treated thylakoids or DCMU-inhibited thylakoids, this result led them to conclude that Q_A^- reoxidation occurred by a back reaction in both cases. (3) Jursinic and Stemler [37] measured Q_A^- reoxidation in thylakoids that had been depleted of bicarbonate. They observed a half-time of 1–2 s in 45% of the complexes and concluded that electron transfer from Q_A to Q_B was impaired. While the correlation between the reoxidation half-time of Q_A^- in the inhibited thylakoids and in the inactive Photosystem II complexes in control thylakoids raises the possibility of a single back-reaction mechanism, the possibility remains that the similarity in half-time is coincidental and the reoxidation of Q_A^- in the inactive fraction occurs by a different pathway.

The extent to which the inactive Photosystem II complexes described here overlap with PS II_β complexes is not easy to answer. Our results indicate the inactive fraction comprises approx. 32% of the total Photosystem II complement. Quantifying the amount of PS II_β is difficult because the slow rise in the fluorescence induction does not reach a clear asymptote: estimates of the amount of PS II_β range from 15 to 35% in spinach thylakoids. Therefore, it is possible that the inactive complexes discussed here and PS II_β centers are identical, likewise, the possiblity exists that the two groups simply overlap, or that PS II_β is a subset of the inactive complexes.

A corollary of identifying the inactive complexes with PS II_β is that, since electron transfer from P-680 to Q_A gives rise to a gramicidin-sensitive absorbance change at 518 nm, the electron-transfer reaction in PS II_β is electrogenic. This conclusion is in disagreement with an earlier suggestion by Van Gorkom that electron transfer from P-680 to Q_A in PS II_β centers is not electrogenic [2,38].

The physical difference between active and inactive complexes is unknown. One possibility is that the inactive complexes do not have plastoquinone in the Q_B site. In this model plastoquinone may occasionally bind to the site but the on-rate can be no faster than a few seconds,

otherwise Q_A^- reoxidation would also be faster. This suggestion is similar to the proposal by Melis [9] that PS II_β complexes are not able to interact freely with the plastoquinone pool. An important element in considering this model is that Graan and Ort have shown the affinity of the Q_B-site for terbutyrin is virtually the same for active and inactive complexes and that the H_2O oxidizing complex is active if an appropriate electron acceptor is present [10]. One possible difference between active and inactive complexes is the redox state of cytochrome *b*-559. We find that there are two high-potential cytochrome *b*-559 hemes per Photosystem II [14]. In addition, there is a variable amount of low potential cytochrome *b*-559 that may be associated with the inactive complexes. Lastly, it is noteworthy that we found that only 70% of the Photosystem II complexes in thylakoids exhibited Q_{400}, as measured by fluorescence induction, and furthermore that the interaction between Q_A and the Fe^{2+} atom exhibits a heterogeneous behavior [39–41]. These observations raise the possibility that the inactive complexes may not exhibit a Q_{400} signal in the presence of ferricyanide due to an altered Q_A-Fe^{2+} interaction, which could account for the altered $Q_A - Q_B$ interaction.

One outgrowth of the work identifying inactive complexes with PS II_β centers is that the disagreement in the literature over the amount of Photosystem II can be resolved (e.g. Ref. 14 and 9). In brief, the measurements of Whitmarsh and Ort showing 1 PS II: 570 Chl * detected only those centers capable of evolving oxygen with dimethylquinone as the acceptor. Graan and Ort have shown that dimethylquinone and the endogenous acceptor plastoquinone are not able to accept electrons from the inactive complexes at a rapid rate [10]. However, probes of Photosystem II that require only a single turnover, or a slow turnover (less than 0.1 e^-/s, e.g., fluorescence induction, Q_A reduction in the presence of DCMU) will detect all of the PS II, active and inactive. Since the inactive complexes make an insignificant contribution to energy transduction it is important when estimating the amount of electron flow due to Photosystem II to determine the active fraction.

Whether there is a significant fraction of inactive complexes in vivo is a question that remains to be explored.

* Calculated according to chlorophyll determined by Ziegler and Egle [15].

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References

1 Diner, B.A. (1986) in Photosynthesis III, Encyclopedia of Plant Physiology (Staehelin, and Arntzen, C.J., eds.), pp. 422–436, Springer-Verlag, Berlin
2 Van Gorkom, H.J. (1985) Photosynth. Res. 6, 97–112
3 Crofts, A.R. and Wraight, C.A. (1983) Biochim. Biophys. Acta 726, 149–185
4 Boussac, A. and Etienne, A. (1982) Biochim. Biophys. Acta 682, 281–288
5 Thielen, A.P.G.M. and Van Gorkom, H.J. (1981) FEBS Lett. 129, 205–209
6 Lavergne, J. (1982) Photobioch. Photobiophys. 3, 257–271
7 Lavergne, J. (1982) Photobioch. Photobiophys. 3, 272–285
8 Selak, M., Koch-Whitmarsh, B. and Whitmarsh, J. (1984) Adv. Photosynth. Res. 1, 493–496
9 Melis, A. (1985) Biochim. Biophys. Acta 808, 334–342
10 Graan, T. and Ort, D.R. (1986) Biochim. Biophys. Acta 852, 320–330
11 Garab, G., Chylla, R. and Whitmarsh, J. (1987) in Hungarian-US Binational Symposium on Photosynthesis (Gibbs, M., ed.), pp. 37–47, Waverly Press, Baltimore, MD
12 Chylla, R., Garab, G., Whitmarsh, J. (1987) in Progress in Photosynthesis Research (Biggins ,J., ed.), Vol. II, pp. 237–240, Martinus Nijhoff, Dordrecht
13 Black, M.T., Brearley, T.H., P. Horton (1986) Photosynth. Res. 8, 193–207
14 Whitmarsh, J. and Ort, D.R. (1984) Arch. Biochem. Biophys. 231, 378–389
15 Ziegler, R. and Egle, K. (9165) Beitr. Biol. Pflanz. 4, 11–37
16 Junge, W. (1984) Curr. Top. Membr. Transp. 16, 431–465
17 Witt, H.T. (1979) Biochim. Biophys. Acta 505, 355–427
18 Meiburg, R.F., Van Gorkom, H.J. and Van Dorssen, R.J. (1983) biochim. Biophys. Acta 724, 352–358

19 Rutherford, A.W. (1985) Photosynth. Res. 6, 295–316
20 Shuvalov, V.A., Nuijs, A.M., Van Gorkom, H.J., Smit, H.W.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 850, 319–323
21 Renger, G. and Wolff, Ch. (1975) Z. Naturofrsch. 30c, 161–171
22 Delosme, R., Zickler, A. and Joliot, P. (1978) Biochim. Biophys. Acta 504, 165–174
23 Junge, W. and Witt, H.T. (1969) Z. Naturforsch. 23b, 244–254
24 Forbush, B. and Kok, B. (1968) Biochim. Biophys. Acta 162, 243–253
25 Robinson, H.H. and Crofts, A.R. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 477–480, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht
26 Bouges-Bocquet, B. (1975) in Proceedings of the 3rd International Congress of Photosynthesis (Avron, M., ed.), pp. 579–588, Elsevier, Amsterdam
27 Diner, B. (1975) in Proceedings of the 3rd International Congress on Photosynthesis (Avron, M., ed.), pp. 589–601, Elsevier, Amsterdam
28 Van Best, J.A. and Duysens, L.N.M. (1975) Biochim. Biophys. Acta 408, 154–163
29 Ikegami, I. and Katoh, S. (1973) Plant Cell Physiol. 14, 829–836
30 Wraight C.A. (1985) Biochim. Biophys. Acta 809, 320–330
31 Petrouleas, V. and B. Diner (1986) Biochim. Biophys. Acta 849, 264–275
32 Melis, A. and Homann, P. (1976) Photochem. Photobiol. 23, 343–350
33 Dennenberg, R.J. and Jursinic, P.A. (1986) Biochim. Biophys. Acta 852, 222–233
34 Renger, G. and Wolff, Ch. (1975) Z. Naturforsch. 30c, 161–171
35 Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357–363
36 Renger, G. and Weiss, W. (1982) FEBS Lett. 137, 217–221
37 Jursinic, P. and Stemler, A. (1982) Biochim. Biophys. Acta 781, 419–428
38 Thielen, A.P.G.M. and Van Gorkom, H.J. (1981) FEBS Lett. 129, 205–209
39 Zimmermann, J.L. and Rutherford, A.W. (1986) Biochim. Biophys. Acta 851, 416–423
40 Petrouleas, V. and Diner, B.A. (1987) Biochim. Biophys. Acta 893, 126–137
41 Diner, B.A. and Petrouleas, V. (1987) Biochim. Biophys. Acta 893, 138–148