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Enhanced oxygen yields caused by double turnovers of Photosystem II induced by dichlorobenzoquinone

Paul A. Jursinic [a,b] and Ronald J. Dennenberg [a]

[a] *Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture *, Peoria, IL and* [b] *Botany Department, University of California, Davis, CA (U.S.A.)*

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The activity of Photosystem II reaction centers increases in the presence of dichlorobenzoquinone (DCBQ) compared to ferricyanide or dimethylbenzoquinone (DMBQ). We demonstrate that enhanced yields from Photosystem II in the presence of DCBQ depend on flash length (temporal distribution). A broad flash with extended decay enhances oxygen yields while a narrow flash without extended decay (300 ns laser flash) does not. The broad flash causes double turnovers of Photosystem II; the narrow flash does not. Double turnovers of Photosystem II oxygen evolution occur in $t \geq 5$ μs specifically in the presence of DCBQ and do not contribute to increased electrochromic absorption change. The maximal enhancement by DCBQ occurs at pH 6.5 and a DCBQ-to-chlorophyll ratio of 1:1. The phase of oscillations in 140 μs delayed fluorescence is advanced by DCBQ as expected of double turnovers in Photosystem II reaction centers. We conclude that DCBQ is not accepting electrons from normally inactive Photosystem II reaction centers but is facilitating double turnovers of normally active reaction centers. If care is taken to avoid double turnovers, the concentration of Photosystem II reactions centers active in oxygen evolution is 1.9 mmol/mol chlorophyll in peas.

Introduction

Photosynthesis takes place through cooperative photoreactions, Photosystems I and II, that act in series to transfer electrons from water to carbon dioxide. An accurate value for the Photosystem II/I ratio is important in devising useful models of photosynthesis. Many different values of Photosystem II/I stoichiometry have been reported [1–7]. Some represent real variation between different plants. Others, we believe [6], are due to errors in determining the concentration of Photosystem II reaction centers that are active in photosynthesis.

* The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Abbreviations: BMBQ, 2-bromo-6-methyl-*p*-benzoquinone; Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCIP, 2,6-dichloroindophenol; DMBQ, 2,5-dimethyl-*p*-benzoquinone; Mes, 4-morpholineethanesulphonic acid; P-680, reaction center of Photosystem II; P-700, reaction center of Photosystem I; Q_a, primary quinone acceptor of Photosystem II; Tes, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulphonic acid; Z, primary electron donor to P-680.

Correspondence: P.A. Jursinic, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604, U.S.A.

The concentration of Photosystem I is not disputed among various research groups and is generally determined by measuring change in absorption at 705 nm, which corresponds to the oxidation of P-700. Several methods, however, have been used to determine Photosystem II. One, which measures quinone reduction as light-induced absorption change at 325 nm in the presence of ferricyanide, consistently yields Photosystem II concentrations of approx. 3 mmol/mol Chl (330 Chl per Photosystem II) [3,5,7,8]. Herbicide binding sites believed to be specific to Photosystem II also number about 3 mmol/mol Chl for atrazine [6,7], terbutryn [9] and DCMU [10]. Measurements of oxygen evolution [6,7,11], proton evolution [5,6], DCIP reduction [6], and cytochrome *b*-559 [5] give Photosystem II concentrations of approx. 1.7 mmol per mol Chl (590 Chl per Photosystem II). A measurement of the electron donor, Z, by ESR techniques [12], which gave 2.5 mmol per mol Chl, is of a questionable value as an independent determination of oxygen-evolving Photosystem II centers because the Z-to-P-680 ratio [12] was based on an assumed value [13] of 400 Chl per P-680.

A number of proposals have sought to improve estimates of Photosystem II concentration. Whitmarsh and Ort [5] suggested that the absorption change at 325 nm was too large due to ultraviolet changes associated with S-state transitions in the oxygen-evolution system [14,15], but significant S-state contributions to the 325 nm absorption change were not observed in later work [6–8,11]. Alternatively, Dennenberg and Jursinic [7] proposed that the absorption change at 325 nm was too large due to photoreduction of a quinone other than Q_a. This quinone, which could be reduced only on the first flash after a dark incubation in the presence of ferricyanide, was originally identified as Q_{400} [7] but is now [16] known to be different from Q_{400} because it has an $E_{m,7} = 318$ mV. Studies of ferricyanide and DCBQ concentration effects indicated that measurements were conducted under optimum conditions [11] and it is not likely that the extra quinone is associated with a reaction center damaged during sample preparation or inactivated by non-physiological electron acceptors [8]. Dennenberg et al. [11] also found a quantum requirement of 9.6 for oxygen evolution, which would be inconsistent with excessive inhibition from sample preparation or added electron acceptors. Inhibition of centers would not cause a change in quantum yield if energy only migrated from closed (inhibited) to open reaction centers. However, as suggested recently [17], energy does not preferentially migrate from closed (inactive) to open (active) reaction centers. In addition, we [11] developed a steady-state flash absorption technique that eliminated the extra quinone absorption found on the first flash in the presence of ferricyanide and thereby reconciled 325 nm absorption changes with oxygen flash yield data.

Yet another explanation for discrepancies in Photosystem II concentration has been suggested by Graan and Ort [9], who found that proton evolution under flash excitation was increased by 50% in the presence of DCBQ and BMBQ but not DMBQ or ferricyanide. They explained this result by proposing the existence of normally inactive Photosystem II reaction centers that become active in electron transport only when DCBQ is present. These data disagree with earlier results that show no enhanced evolution of oxygen [6,7,11] or protons [6] when DCBQ is present.

In this work, we demonstrate that enhanced yields of Photosystem II in the presence of DCBQ depend on flash length (temporal distribution). We find that a broad flash with extended decay shows enhancement of oxygen yields by DCBQ while a narrow flash without extended decay (300 ns laser flash) does not. The broad flash causes double turnovers of Photosystem II while the narrow flash does not. Furthermore, the phase of oscillations in 140 μs delayed fluorescence is advanced in the presence of DCBQ, as would be expected if double turnovers in Photosystem II reaction centers were occurring. We conclude that DCBQ is not accepting electrons from normally inactive Photosystem II reaction centers but is facilitating multiple turnovers of reaction centers on each flash.

Materials and Methods

Spinach (*Spinacia oleracea* L.) was purchased from the local market. Dwarf pea seedlings (*Pisum sativum* L. var. Wando) were grown in vermicu-

lite-filled trays in a growth chamber (16 h day; 25/20 °C; 70 W/m^2 light intensity from a combination of cool-white fluorescent and incandescent lamps). The plants were harvested 18–21 days after germination. Thylakoids (broken chloroplasts) were isolated from leaves as described previously [18]. Both fresh and frozen samples were used. Fresh samples were used within 3 h of isolation. Frozen samples were stored in liquid nitrogen until used. Reaction media contained: 400 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and, in the pH 7.3–8.5 range, 50 mM Tes or, in the 6–7.2 range, 50 mM Mes.

Oxygen yield per flash was determined by a Clark-type electrode as described previously [6]. The number of Photosystem II reaction centers connected to active oxygen-evolving complexes was calculated by multiplying the oxygen flash yield by 4, since on any flash only one-fourth of the reaction centers evolve oxygen [19].

Flash excitation for oxygen evolution was provided by a number of different excitation sources. A Phase-R model DL-1100 dye laser was used with LD490 dye having a lasing wavelength of 479 nm and a pulse width at half maximum of 300 ns [20]. EG&G model FX200, xenon, strobe lamps were operated at 1500 V with 4 μF capacitance (4.5 J input energy) and no optical filtering. Red flashes were generated by running the EG&G lamp at 1500 V with 8 μF capacitance (9.0 J input energy) and filtered by a Corning CS 2-62 glass filter. Both types of flashes were able to saturate the oxygen photoreaction with ferricyanide as the electron acceptor. This was demonstrated by a negligible decrease in signal size when a 70% transmission neutral density filter was inserted in the light path. For the data in Fig. 2 two General Radio model 1538-A xenon flash lamps were also used.

The time dependence of the flash emission was measured by a reverse-biased United Detector Technology PIN020A photodiode that had a 5 ns electronic risetime. The analog signal of the photodiode was digitized with a Biomation model 2805 waveform recorder that had a 127 ns risetime and 200 ns digitization rate. The digitization rate, the time-limiting factor in this measuring system, was significantly faster than the microsecond flash emission.

Photochemically induced absorption changes at 325, 705 and 515 nm were measured with a laboratory-built spectrophotometer [7]. Delayed fluorescence was measured as previously described [21] except excitation was provided by two General Radio model 1538-A xenon flash lamps filtered by Corning CS 4-96 glass filters.

Ferricyanide was purified by recrystallization from solution in water. DCBQ and DMBQ were purchased from Eastman Kodak Company and purified by sublimation.

Results

Observations by Graan and Ort [9] that proton evolution under flash excitation is 50% greater with DCBQ as electron acceptor than with DMBQ or ferricyanide disagree with our earlier work in which we found no enhanced evolution of oxygen [6,7,11] or protons [6] when DCBQ was the acceptor. This discrepancy might be explained if under their conditions DCBQ rapidly reoxidized a Photosystem II acceptor making possible multiple turnovers of Photosystem II. Multiple turnovers would occur if the flash decay were comparable to the reoxidation time of a Photosystem II acceptor in the presence of DCBQ and if there were sufficient energy in the tail of the flash.

Fig. 1 shows the time dependence of light absorbed by thylakoids from the various xenon flash lamps. The General Radio lamps provide about one-third the peak irradiance of the EG&G lamp with a width at half height of 2.5 μs. The red filtered EG&G lamp has a slower rise and lengthened decay tail to give a width at half height of 8 μs. This broadening of the flash profile is caused by the larger storage capacitor to provide higher energy and by the red optical filtering, which emphasizes the shift to red emission of the decay tail.

The effects of various light sources on oxygen flash yields are shown in Table I. Results with the laser and single EG&G flash confirm our earlier results [6,7,11]. That is, in thylakoids ferricyanide, DMBQ, and DCBQ all give the same oxygen yield per flash. DCBQ gives an enhanced yield of oxygen only if sufficient energy is available in the decay tail of the flash ($t > 5$ μs). This is clear when one compares a laser flash which has 40-times more

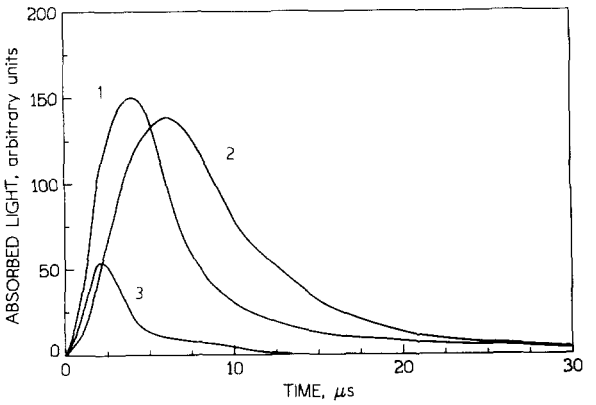

Fig. 1. Time dependence of light absorbed by pea thylakoids from various flash lamps. Curves 1, 2 and 3 are for one EG&G flash, one red flash and two General Radio flashes, respectively, in Materials and Methods. All of the flashes are triggered at $t = 0$ and the intensities are shown unnormalized. Measurements were made through a 1 cm pathlength cuvette filled with buffer to give the incident light and filled with 50 μg Chl/ml sample to give the transmitted light. The time profile of the absorbed light was calculated as the difference between the incident and transmitted light.

energy than two EG&G lamps. With the laser, the energy is delivered in 300 ns and no DCBQ enhancement is observed. However, when two EG &G flashes are given simultaneously ($\Delta T_{12} = 0$) or are separated by 30 μs ($\Delta T_{12} = 30$ μs) or a red flash is given, then sufficient energy is delivered in the microsecond range of the decay tails to support DCBQ enhancement. These experiments were also carried out on freshly prepared thylakoids and similar results were found (data not shown). This finding that the DCBQ enhancement of oxygen yield depends on the length of the excitation flash is consistent with our hypothesis of multiple turnovers of Photosystem II during a flash, and it is inconsistent with the inactive-center hypothesis [9]. Based on the flash profiles of Fig. 1, the reoxidation of a Photosystem II acceptor by DCBQ must be occurring in the range of 5 μs or longer.

If our multiple-turnover hypothesis is correct, such turnovers could be induced by one EG&G flash followed with a few-microsecond separation by a simultaneously triggered pair of General Radio lamps. Referring to Fig. 1, this is equivalent to shifting flash 3 along the time axis of flash 1. The use of two flashes in this manner allows the char-

TABLE I

COMPARISON OF OXYGEN-EVOLUTION CAPACITY WITH NARROW AND BROAD EXCITATION FLASHES

All measurements were made at a Chl concentration of 50 μg/ml with 0.5 μM gramicidin present. Thylakoids that were stored at 77 K were used. The various flashes are described in Materials and Methods and Fig. 1. ΔT_{12} is the separation time between the two EG&G flashes. The incident light energy provided by the laser was 160 mJ·cm^{-2} per flash and by one EG&G lamp was 2 mJ·cm^{-2} per flash. Values are in mmol O_2 per mol Chl per flash. A standard deviation of 5% is found for measurements made on five different vials of frozen thylakoids. Note that 490 mmol O_2 per mol Chl per flash is equivalent to 510 Chl flash per electron, assuming four electrons are transferred per oxygen evolved.

Electron acceptor	Laser	One EG&G flash	Two EG&G flashes		Red flash
			$\Delta T_{12} = 0$	$\Delta T_{12} = 30$ μs	
1 mM ferricyanide	497	490	490	490	474
1 mM ferricyanide +100 μM DMBQ	–	474	497	490	490
1 mM ferricyanide +100 μM DCBQ	478	490	614	623	647

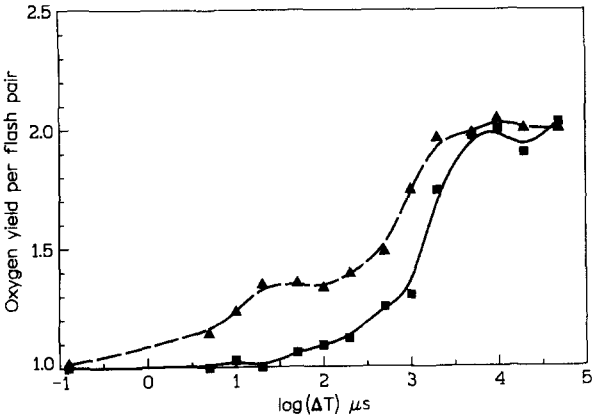

Fig. 2. Yield of oxygen per flash pair as a function of separation time, Δt, between two narrow flashes. Flash pairs were given at 5 Hz. The first xenon flash of each flash pair was an EG&G type described in Materials and Methods and Fig. 1, and the second xenon flash of the flash pair was a simultaneously triggered pair of (General Radio 1538-A) flash lamps. Measurements were made at a Chl concentration of 50 μg/ml in pH 6.5 buffer with 1 mM ferricyanide (■———■) or 1 mM ferricyanide + 100 μM DCBQ (▲------▲) present as electron acceptor. Acceptors were added in the dark 2 min prior to the start of flash excitation. An ordinate value of 1.0 is equivalent to 513 mmol O_2 per mol Chl per flash or 487 Chl flash per electron.

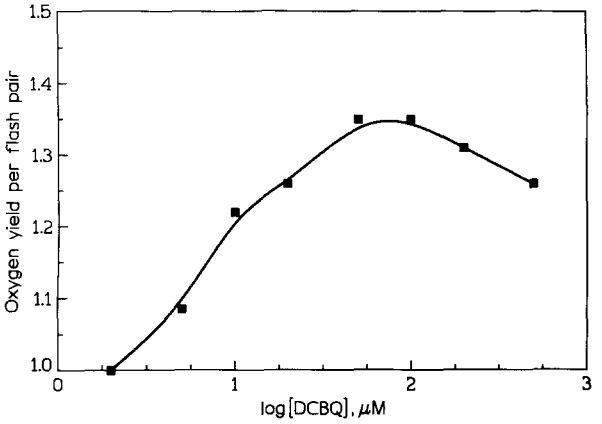

Fig. 3. Yield of oxygen per flash pair as a function of DCBQ concentration. Separation time between the two flashes of each flash pair was 30 μs. DCBQ was added 2 min prior to the start of flash excitation. Other conditions were as described in the legend of Fig. 2.

acterization of the time dependence of the DCBQ enhancement. Fig. 2 shows the effect of varying the separation time between flashes. Enhancement of the oxygen yield, which is specific to DCBQ, is observed with a separation of 5 μs or more between the flashes. This is consistent with the multiple-turnover hypothesis. The first flash of the pair energizes Photosystem II charge separation and reduces all the electron acceptors. Within 20 μs, in the presence of DCBQ but not ferricyanide, a Photosystem II acceptor is reoxidized, which allows the second flash of the pair to energize an additional Photosystem II charge separation. This is observed as enhanced oxygen evolution in Fig. 2 at Δt between 5 and 20 μs.

Fig. 3 is the dependence of this enhancement of oxygen yield per flash on DCBQ concentration. A maximum enhancement is reached at 50 μM DCBQ (DCBQ/Chl = 1 : 1), which is in reasonable agreement with earlier work (DCBQ/Chl = 1 : 2) [9]. The DCBQ enhancement decreases at concentrations of at least 200 μM, which corresponds with this quinone's inhibition of Photosystem II activity at high concentrations [11].

It was stated without data [9] that DCBQ inhibited flash-induced water oxidation at pH > 7. In Fig. 4 we show the effect of pH on oxygen evolution excited by a pair of flashes given simultaneously or with 30 μs separation. When a simultaneous pair of flashes is used (■——■), flash yields of oxygen are maximal from pH 6.5 to 7.5. We found no significant inhibition immediately above pH 7, but found inhibition at pH < 6.5 or pH > 7.7. When a flash pair separated by 30 μs is used (▲——▲) oxygen yield enhancement is maximal at pH 6.5 and vanishes at pH > 7.7. Clearly, pH 6.5 is the best condition for observing double turnovers of Photosystem II in the presence of DCBQ and a broad flash.

The possibility of double turnovers was considered by Graan and Ort [9] and tested by observing the electrochromic absorption change (Abs 518–540 nm) with a broad xenon and narrow laser flash. They assumed, but did not demonstrate, that electrochromism was proportional to the DCBQ enhancement phenomenon they were observing. Electrochromic absorption change would be invalid as a test for double turnovers if it were insensitive to the DCBQ enhancement of Photosystem II. In Fig. 5A we measured the electrochromic absorption change under conditions that we found maximized the DCBQ enhancement of water oxidation; i.e., two EG&G lamps with flashes separated by 30 μs, red filtering, 50 μM DCBQ, and 500 μM FeCN. The absorption change decreased by one-third when DCBQ was present

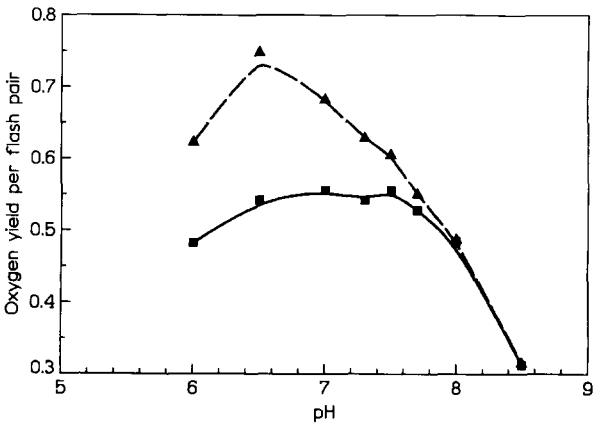

Fig. 4. Yield of oxygen per flash as a function of reaction pH. Measurements were made with flash pairs, which consisted of one EG&G flash and two simultaneously triggered General Radio flashes separated by zero (■——■) or 30 μs (▲------▲). Flash pairs were given at 5 Hz. The electron acceptor system was 1 mM ferricyanide and 100 μM DCBQ. Thylakoids that were stored at 77 K were used. An ordinate value of 0.55 is equivalent to 500 mmol O_2 per mol Chl per flash or 500 Chl flash per electron.

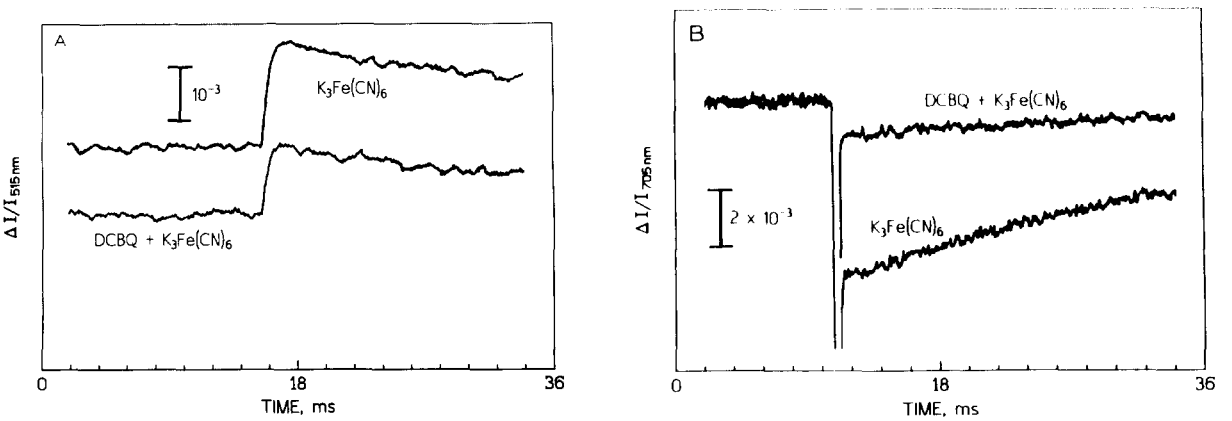

Fig. 5. (A) Gramicidin sensitive portion of the absorption change at 515 nm in fresh pea thylakoids induced by two saturating flashes separated by 30 μs. Each trace is the average of 200 flash trains delivered at 1 Hz. The Chl concentration was 25 μg/ml; reaction medium, pH 6.5; electronic rise-time, 1 ms. (B) Absorption change at 705 nm in pea thylakoids. Each trace is the average of 600 flashes delivered at 2 Hz. Chl concentration was 25 μg/ml; reaction medium, pH 6.5; electronic rise-time, 100 μs.

compared to when it was absent. This result is surprising if one presumes that DCBQ enhancement is electrochromic and if one considers no other changes in photochemistry. However, the data can be explained by the following hypothesis and experimental results. In the absence of DCBQ, both Photosystem II and Photosystem I are turning over on each flash, since ferricyanide is primarily a Photosystem I electron acceptor. Upon the addition of DCBQ, Photosystem I charge separation is largely suppressed as P-700 and the plastoquinone pool are oxidized after the first flash by the DCBQ-ferricyanide couple [11,22]. In this sample, we measured a decrease of 85% in Photosystem I activity (see Fig. 5B) with the addition of DCBQ. We can then calculate a Photosystem II/I ratio as follows:

$$\Delta I/I \text{ 515 nm (FeCN)} = \text{PS II} + \text{PS I} \quad (1)$$

$$\Delta I/I \text{ 515 nm (FeCN, DCBQ)} = \text{PS II} + 0.15\ \text{PS I} \quad (2)$$

It is assumed the contributions to electrochromism are proportional to charge separation at Photosystems II and I [23], and do not have a component sensitive to DCBQ enhancement. Solving the equations simultaneously we calculate a Photosystem II/I ratio of 1.56. A Photosystem II/I ratio of 1.66 was determined independently based on measurements of absorption change at 325 nm (2.44 mM quinone per mol Chl) and 705 nm (1.47 mM P-700 per mol Chl) using methods reported previously [11]. The close agreement between these two determinations of the Photosystem II-to-I ratio

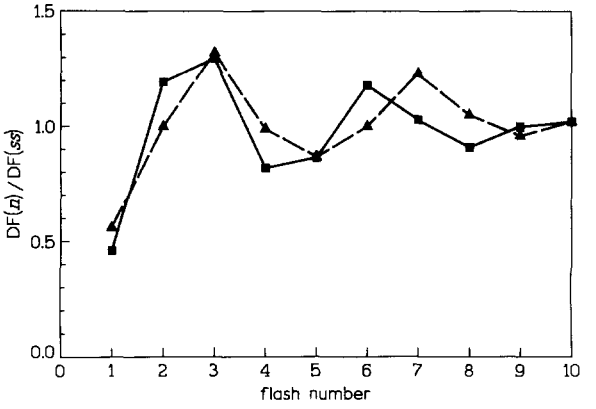

Fig. 6. Amplitude of delayed fluorescence (DF) as a function of flash number in the presence (■———■) and absence (▲------▲) of DCBQ and ferricyanide. Flash excitation provided by a pair of General Radio flashes separated by 30 μs. Flash pair were given at 2 Hz. Delayed fluorescence amplitude was measured 140 μs after the last flash of the flash pair. Thylakoids that were stored at 77 K were measured in pH 6.5 reaction medium at a Chl concentration of 10 μg/ml. Samples were dark adapted for 10 min and DCBQ was added 2 min before start of the measurement. The DCBQ concentration was 20 μM and ferricyanide was 100 μM, which was the same DCBQ/Chl ratio and ferricyanide/Chl ratio as in Figs. 2, 4 and 5. DF(ss) was the steady state value reached after 20 flashes. DF(ss) was 25% lower in the presence of DCBQ, which was approximately the amount of DCBQ quenching of the o-level of Chl *a* fluorescence.

is consistent with the suggestion that the electrochromic absorption change is insensitive to DCBQ-induced double turnovers (our hypothesis) or the DCBQ-oxidation of inactive Photosystem II (Graan and Ort hypothesis). Electrochromic experiments that used only one EG&G flash lamp (data not shown) gave the same absorption change as those that used two lamps with a 30 μs flash separation (Fig. 5). We conclude that electrochromism is not sensitive to DCBQ enhancement and, therefore, not a useful test for double turnovers.

We sought other methods to test for double turnovers in Photosystem II. The pattern of oxygen yield vs. flash number is a method that has been used to describe double turnovers in Photosystem II reaction centers [16,19,20,24]. Attempts to measure oxygen flash yields were unsuccessful. DCBQ reacted directly with the bare platinum electrode resulting in large flash-induced signals that masked oxygen evolution signals.

Another phenomenon that is sensitive to S-state advancement and double turnovers in photosystem II is microsecond delayed fluorescence [25–28]. Fig. 6 shows the flash pattern of delayed fluorescence in the presence and absence of DCBQ with broad-flash conditions provided by a flash pair separated by 30 μs. The flash pattern without DCBQ shows the typical period-4 oscillation with maxima on flashes 3 and 7. When DCBQ is present, the flash pattern is advanced in phase, which is most easily seen by the larger delayed fluorescence on flash 2 compared to the control and the maximum on flash 6 instead of flash 7. This behavior in the presence of DCBQ is consistent with double turnovers at the Photosystem II reaction center.

Discussion

In earlier work, Dennenberg et al. [11] found that the oxygen flash yield in thylakoids was unchanged using different acceptor systems. It was also found that the quantum requirement of oxygen evolution of typical thylakoid preparations from both peas and spinach approached 90% of the theoretical value. The observations of Graan and Ort [9] that DCBQ increased H^+ evolution by 50% led them to postulate that fully one-third of the PS II reaction centers were normally inactive. Their observations conflicted with our data and could not readily be reconciled to our quantum requirement measurement. In this work we sought an explanation that would encompass the observations of Graan and Ort [9]. We propose that multiple turnovers of photosystem II can occur in the presence of DCBQ if the flash is sufficiently broad. We also believe that it is unnecessary to invoke a population of Photosystem II centers that is normally inactive except in the presence of DCBQ.

Normally with flashes of a few microseconds duration, double turnovers of Photosystem II occur with low probability [19]. This is due to long times for charge transfer; in the range of 100 μs to 1 ms [29,30] on the donor side of P-680; in the 400 μs range [31] on the acceptor side of P-680. The limitation on the acceptor side can be alleviated for one flash after 5 min incubation in the dark with ferricyanide [20,24,32]. This dark incubation with ferricyanide oxidizes a secondary acceptor, Q_{400}, that can accept electrons from Q_a^-. We propose that ferricyanide with DCBQ as a redox mediator also oxidizes Q_{400} but in less than 50 ms instead of 5 min as with ferricyanide alone. In the presence of DCBQ, Q_{400} will be oxidized between flashes and limitations on the acceptor side of P-680 will be alleviated on every flash.

The data presented here can be more easily understood by referring to the reaction scheme of Fig. 7. In the presence of DCBQ, Q_a and Q_{400} will be oxidized between flashes. Excitation with a single narrow flash will only activate reaction 1, and reactions 2, 3 and 4 will take place in 25 ns to 1 ms [29,30,33,34]. Photoreaction 5 will only occur with a long flash. With a narrow flash the single advancement in S-states ($S_n \rightarrow S_{n+1}$) by reactions 1–4 will be highly favored over the double advancement ($S_n \rightarrow S_{n+2}$) by reactions 1, 2, 3, 5 and 6. This explains the data in Table I and the lack of DCBQ enhancement of oxygen evolution [6,7,11] when a laser or a single EG&G flash is used.

Reaction 5 of Fig. 7 only occurs if quanta are available for a time sufficient for reaction 3 to take place. This is accomplished with a broad flash (a flash with sufficient intensity in its decay tail) or a pair of narrow flashes separated by an appropriate time, Δt. Under these conditions, the reaction path of 1, 2, 3, 5 and 6 will occur and give

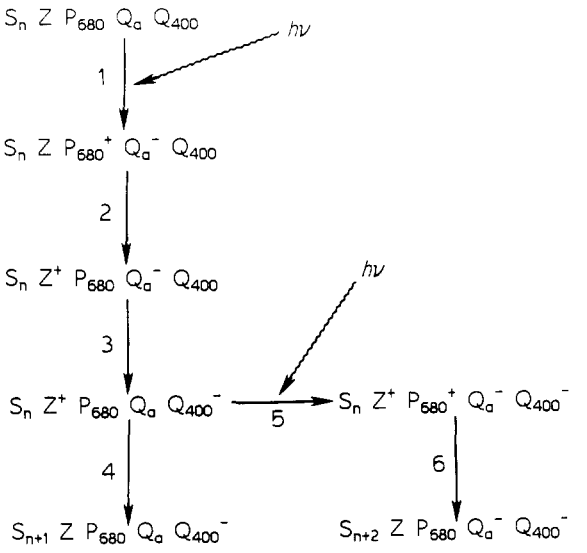

Fig. 7. Reaction scheme for the Photosystem II reaction center that includes Q_a and Q_{400}. S_n are S-states of the oxygen evolution system. Reactions 1 and 5 are photoreactions. Reaction 2 occurs in less than 400 ns [37]. Other details are given in the Discussion section.

rise to double advancement in S-states ($S_n \rightarrow S_{n+2}$). This is seen as DCBQ-enhanced proton evolution [9] and oxygen evolution (Table I) when two EG&G flashes or a red flash is used. Two narrow flashes separated by $\Delta t \geq 5$ μs (Fig. 2) also support DCBQ enhancement of oxygen evolution. A kinetic limitation for oxygen evolution in the range of 5 μs has been observed previously [20]. Based on the data of Fig. 2 we would estimate the lifetime of reaction 3 to be 5 μs or more, which is smaller than the value of 25 μs measured in thylakoid membrane fragments [33]. Preliminary measurements in our laboratory indicate this is due to a difference between thylakoids and membrane fragments.

Even in the presence of DCBQ a broad flash does not result in 100% double turnovers of Photosystem II (Table I, Figs. 2–4). This is probably due to rate limitations in charge transfer on the donor side of P-680. The S_0Z^+P-680 → S_1ZP-680 and S_1Z^+P-680 → S_2ZP-680 reactions occur in less than 100 μs, the S_2Z^+P-680 → S_3ZP-680 reaction occurs in 500 μs, and the S_3Z^+P-680 → S_4ZP-680 → S_0ZP-680 oxygen evolution reaction occurs in 1 ms [29,30]. Another explanation for the low number of double turnovers is a competition between reaction 6 (Fig. 7) and recombination between P-680$^+Q_a^-$ following reaction 5. Also, it has been reported [33] that all Q_{400}'s are not oxidizable or a large number of Photosystem II reaction centers do not have Q_{400}.

The consequence of Photosystem II double turnovers is shown in Fig. 6 as a shift in the phase of oscillations in 140 μs delayed fluorescence in the presence of DCBQ. Such an effect is not predicted by the inactive center hypothesis [9].

For measurements of proton evolution, Graan and Ort [9] used two red-filtered flashes each with profiles similar to the EG&G flash used in this work (personal communication between Jursinic and Ort). As shown in Column 4 of Table I, two EG&G flashes have sufficient energy at 5 μs and longer to support double hitting in oxygen evolution when DCBQ is present. While we cannot be certain of the experimental conditions of Graan and Ort [9], we believe our hypothesis of double hitting in Photosystem II is a reasonable alternative to their suggestion of 40% inactive centers. While these double turnovers enhance oxygen evolution (Table I, Fig. 2–4) they are not electrochromic (Fig. 5). We have no explanation for this lack of contribution to membrane potential. However, charge separation in Photosystem II that is not electrochromic has been reported previously. A secondary electron acceptor known as Xa supported charge separation [35] but did not give rise to oxygen evolution or electrochromism [36]. Another acceptor known as Q_2 can participate in oxygen evolution under very high flash intensity [37] but it is not electrochromic [38]. However, since neither Xa nor Q_2 required the presence of DCBQ, they apparently are not related to phenomena reported here.

In thylakoids, with methyl viologen as an acceptor, a component of this electrochromic absorption change at 515 nm was found to recover with a 3 s half time [39]. They hypothesized that this was due to 30–38% of Photosystem II reaction centers being unconnected to the plastoquinone pool. These centers were suggested to be identical to what Graan and Ort [9] believed were enhanced by DCBQ. From our results in Fig. 5, the DCBQ enhancement of oxygen evolution is not electrogenic and so cannot be the explanation for this slow recovery 515 nm absorption change.

We conclude that if care is taken to avoid double turnovers the concentration of Photosystem II reaction centers that are active in oxygen evolution is 1.9 mmol per mol Chl (525 Chl per Photosystem II). Double turnovers are favored under the following conditions: presence of DCBQ and ferricyanide, pH 6.5, and excitation with flashes that have significant emission at 5 μs or longer.

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References

1 Fujita, Y. (1976) Plant Cell Physiol. 17, 187–191.
2 Kawamura, M., Mimuro, M. and Fujita, Y. (1979) Plant Cell Physiol. 20, 697–705.
3 Melis, A. and Brown, J.S. (1980) Proc. Natl. Acad. Sci. USA 77, 4712–4716.
4 Meyers, J. and Graham, J.R. (1983) Plant Physiol. 71, 440–442.
5 Whitmarsh, J. and Ort, D.R. (1984) Arch. Biochem. Biophys. 231, 378–389.
6 Jursinic, P. and Dennenberg, R. (1985) Arch. Biochem. Biophys. 241, 540–549.
7 Dennenberg, R. and Jursinic, P. (1985) Biochim. Biophys. Acta 808, 192–200.
8 McCauley, S.W. and Melis, A. (1986) Biochim. Biophys. Acta 849, 175–182.
9 Graan, T. and Ort, D.R. (1986) Biochim. Biophys. Acta 852, 320–330.
10 Graan, T. (1986) FEBS Lett. 206, 9–14.
11 Dennenberg, R.J., Jursinic, P.A. and McCarthy, S.A. (1986) Biochim. Biophys. Acta 852, 222–233.
12 Babcock, G.T., Ghanotakis, D.F., Ke, B. and Diner, B.A. (1985) Biochim. Biophys. Acta 723, 276–286.
13 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.
14 Thielen, A.P.G.M. and Van Gorkom, H.J. (1981) Biochim. Biophys. Acta 637, 439–446.
15 Dekker, J.P., Van Gorkom, H.J., Wensink, J. and Ouwenhand, L. (1984) Biochim. Biophys. Acta 767, 1–9.
16 Jursinic, P. and Dennenberg, R. (1988) Biochim. Biophys. Acta, Vol. 935, in press.
17 Ley, A.C. and Mauzerall, D.C. (1986) Biochim. Biophys. Acta 850, 234–248.
18 Jursinic, P. (1978) FEBS Lett. 90, 15–20.
19 Joliot, P. and Kok, B. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 387–412, Academic Press, New York.
20 Jursinic, P. (1981) Biochim. Biophys. Acta 635, 38–52.
21 Jursinic, P. and Stemler, A. (1982) Biochim. Biophys. Acta 681, 419–428.
22 Junge, W. and Ausländer, W. (1973) Biochim. Biophys. Acta 333, 59–70.
23 Schliephake, H., Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23b, 1571–1578.
24 Velthuys, B. and Kok, B. (1978) in Proceedings of the 4th International Congress on Photosynthesis (Hall, D.O., Coombs, J. and Goodwin, T.W., eds.), pp. 397–407, Ballantyne Press, London.
25 Zankel, K. (1971) Biochim. Biphys. Acta 245, 373–385.
26 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 223–317, Academic Press, New York.
27 Wydrzynski, T., Higgins, B.J. and Jursinic, P.A. (1985) Biochim. Biophys. acta 809, 125–136.
28 Jursinic, P.A. (1986) in Light Emission by Plants and Bacteria (Govindjee, Amesz, J. and Fork, D.C., eds.), pp. 291–328, Academic Press, New York.
29 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 292, 772–785.
30 Babcock, G.T., Blankenship, R.E. and Sauer, K. (1976) FEBS Lett. 61, 286–289.
31 Mauzerall, D. (1972) Proc. Natl. Acad. Sci. USA 69, 1358–1362.
32 Ikegami, I. and Katoh, S. (1973) Plant Cell Physiol. 14, 829–836.
33 Petrouleas, V. and Diner, B.A. (1987) Biochim. Biophys. Acta 893, 126–137.
34 Brettel, K., Schlodder, E. and Witt, H.T. (1984) Biochim. Biophys. Acta 766, 403–415.
35 Eckert, H.J., Buchwald, H.E. and Renger, G. (1979) FEBS Lett. 103, 291–295.
36 Eckert, H.J. and Renger, G. (1980) Photochem. Photobiol. 31, 501–511.
37 Joliot, P. and Joliot, A. (1981) Biochim. Biophys. Acta 638, 132–140.
38 Joliot, P. and Joliot, A. (1981) FEBS Lett. 134, 155–158.
39 Chylla, R., Garab, G. and Whitmarsh, J. (1987) Biochim. Biophys. Acta 894, 562–571.