*Biochimica et Biophysica Acta*, 1101 (1992) 1–12




BBABIO 43625

# Plastoquinone compartmentation in chloroplasts. I. Evidence for domains with different rates of photo-reduction

Pierre Joliot, Jérôme Lavergne and Daniel Béal

*Institut de Biologie Physico-Chimique, Paris (France)*

(Received 16 October 1991)
(Revised manuscript received 11 March 1992)

Key words: Plastoquinone; Electron transfer chain; Photosystem II; Plastocyanin; Restricted diffusion; Grana; Stroma

The photo-reduction of plastoquinones by Photosystem II reaction centers was investigated using fluorescence and oxygen-evolution measurements in thylakoids deprived of Photosystem I acceptors. The process appears biphasic under limiting, as well as saturating, illumination. The 'fast' pool fraction (about 6 PQ molecules per PS II center) represents 50–70% of the total. Its half reduction time under saturating light was found about 25–60 ms, while that of the 'slow' pool was 0.8–1 s. When the photo-reduction process is interrupted after reduction of the fast pool and resumed after a dark period, a redistribution of the reduced plastoquinones towards the slow pool is observed, with $t_{1/2} \approx 6$ s. We interpret these results as expressing a limitation of PQ diffusion in the membrane and propose that the fast pool reflects the fraction present in the grana region where most PS II centers are located, while the slow pool corresponds to quinones from the stromal region. The relationship between the redox states of PQ and of the primary acceptor $Q_A$ during photo-reduction of the fast pool expresses marked discrepancies with respect to a quasi-equilibrium relationship. This failure to achieve equilibrium on a rapid time-scale and the slow diffusion rate of quinones over long distances are accounted for by small size domains bounded by membrane proteins. In agreement with this view, we found that the amount of fast photo-reducible quinones is decreased when a fraction of PS II centers is inhibited, indicating that the domains contain, on average, about 3–4 PS II centers. We conclude that PQ cannot be responsible for the long range diffusion involved in rapid electron transfer from granal (PS II) to stromal (PS I) regions, a role that must be fulfilled by plastocyanin.

## Introduction

The electron transfer chain of chloroplasts involves a pool of lipid-soluble plastoquinone molecules that accept electrons from PS II and deliver them to the cytochrome $b_6/f$ complex; subsequent transfer towards PS I is mediated by another soluble carrier, plastocyanin, that diffuses in the lumenal water phase (see Ref. 1 for a review). The number of plastoquinone molecules per PS II center has been estimated in the range of 5–7 [2–4], thus a pool capacity of 10–14 electrons. It was shown long ago that several chains are connected through a common plastoquinone pool [5,6], suggesting, but not proving, free diffusion of these molecules in the lipid phase of the membrane. The space and time ranges of plastoquinone diffusion have direct bearing on the question of the mechanism of long-distance electron transfer between the two photosystems. Most PS II centers are contained in the stacked granal regions, while PS I centers are all located in the unstacked regions, at a distance of the order of 0.1 $\mu$m [7] (however, the presence of a fraction of PS I in the peripheral margin of the grana has been hypothesized by Albertsson et al [8]). Immunocytochemistry investigations [9,10] have shown that more than half of the cytochrome $b_6/f$ complexes are located in the grana, close to PS II centers. Thus, it seems likely that plastocyanin, that shuttles between this complex and PS I, is involved in long range transfer: otherwise no clear function could be ascribed to the large $b_6/f$ fraction present in the grana. There is, indeed, kinetic evidence showing that the totality of the $b_6/f$ complexes can be photo-oxidized by PS I (through plastocyanin) in a few milliseconds [1,11]. Furthermore, it has been shown that plastocyanin is found in both granal and agranal fractions of the thylakoids [12]. However, these observations do not exclude plastoquinone as a long range carrier, a view which has been advocated by several authors [13,14].

Correspondence: P. Joliot, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France.

Abbreviations: PS I/II, Photosystem I/II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PQ, plastoquinone.

A second important question that remains unsolved is the relationship between the plastoquinones of the pool and the plastoquinone acceptor of PS II, $Q_A$. The mid-point potential of the latter species at pH 7 has been estimated in the −45 to 0 mV range [15–18]. There is not general agreement on the pH dependence of this potential that some authors found to vary by −60 mV per pH unit [15,16], whereas others found a weaker [18] or no [17] dependence. Thus it is not clear whether the $E_{m_7}$ slightly below 0 mV reflects a protonation (presumably indirect) of $Q_A$. On the other hand, it seems likely that no such protonation occurs on a short time-scale since no proton uptake is detectable upon photo-reduction of $Q_A$ in the presence of DCMU [19,20]. If a protonated state is involved in redox titrations, the functionally relevant mid-point potential of $Q_A$ should be that observed at pH > p$K$, thus about −130 mV according to Knaff [16] who found a p$K \approx 8.9$. The mid-point potential of the plastoquinone pool is known to be about +110 mv (pH 7) [21]. Thus the equilibrium constant between $Q_A$ and PQ is at least 70 (and possibly much larger − $10^4$ if Knaff's finding is correct). Accordingly, in the equilibrated system, one expects $Q_A$ to remain oxidized as long as the PQ pool is not almost completely reduced. This situation should be observed at low rates of photo-reduction of the pool so that electron transfer is not kinetically limiting. In sharp contrast with this prediction, the accumulation of $Q_A^-$ is observed [2,22,23] to occur almost simultaneously with the photo-reduction of the pool. The 'apparent equilibrium constant' deduced from such experiments was reported to be in the range of 1–5. In living algae, under weak steady-state illumination at various wavelengths, equal fractions of reduced $Q_A$ and PQ were measured (apparent constant of 1) [2]. In isolated, uncoupled thylakoids, in the absence of a PS I acceptor, Forbush and Kok [22] determined an apparent equilibrium constant of 3 by measuring the redox state of $Q_A$ by fluorescence during photo-reduction of the pool.

These earlier determinations of the 'apparent equilibrium constant' were done before plastoquinones were identified as secondary acceptors and the thermodynamic relationship between $Q_A$ and its acceptors was analyzed in terms of one-electron carriers. PQ is actually a two-electron carrier, which (as illustrated later in Fig. 10 of this paper and in Fig. 1 of the accompanying paper, Ref. 27) changes significantly the shape of the equilibrium relationship. Furthermore, we know that electron transfer between $Q_A$ and PQ is mediated by the two-electron gate $Q_B$. If this is taken into account, one should consider two sets of equilibria. The 'even set' in which the quinone acceptors of the reaction center contain zero or two electrons:

$$Q_A + PQ \rightleftharpoons Q_A Q_B \quad (1)$$

$$Q_A + PQ^- \rightleftharpoons Q_A Q_B^- \quad (2)$$

$$Q_A Q_B^- \rightleftharpoons Q_A^- Q_B \quad (3)$$

and the 'odd set' where the number of electrons is one or three:

$$Q_A^- + PQ \rightleftharpoons Q_A Q_B \quad (4)$$

$$Q_A^- + PQ^- \rightleftharpoons Q_A Q_B^- \quad (5)$$

$$Q_A Q_B^- \rightleftharpoons Q_A^- Q_B \quad (6)$$

where $Q_B$ stands for the PQ bound in the $Q_B$ pocket of the center. These two sets do not equilibrate with one another on a short time-scale. A rigorous discussion of the $Q_A$ − PQ relationship should deal with the light-induced shuttling between these sets and the three equilibria controlling each of them. We need not really go into such details here, for it was shown by Diner [24] that in both sets the equilibria are much displaced towards $Q_A$ oxidation. In the even set, the ratio $[Q_A Q_B^{2-}]/[Q_A^- Q_B^-]$ was estimated greater than 50: thus even at complete pool reduction, $Q_A$ remains almost fully oxidized in this set of states. In the odd set, an equilibrium constant of 15–20 was found by Diner for $[Q_A Q_B^-]/[Q_A^- Q_B]$. The equilibria considered by Diner are not identical with [4–6] since the concept of PQ exchange in the $Q_B$ pocket had not been introduced at that time, but we may use the raw data given in this paper, indicating, e.g., 9% or 31% reduction of $Q_A$ (in excess of the 5% observed when PQ is fully oxidized) at, respectively, 59% or 89% of PQ reduction. Therefore, it is clear that the $Q_B$ gate provides no clue to the problem of the anomalously small apparent equilibrium constant describing the photo-reduction process. For simplicity, we shall generally keep implicit the role of the $Q_B$ gate when discussing the $Q_A$ − PQ relationship.

It is important to stress that the low value found for the apparent equilibrium constant is not modified for a large range of illumination intensities (roughly 2 to 20 photochemical turnovers of PS II per s). Nevertheless, when the system has been dark-adapted for more than 30 s, the redox states of $Q_A$ and PQ are consistent with the large equilibrium constant expected from redox titrations. These results were originally described in terms of a light-induced change of the equilibrium constant, the origin of which remained mysterious [23]. We now argue that a more likely explanation is that full thermodynamic equilibration between $Q_A$ and PQ is a much slower process than the rate of electron transfer between them. In recent papers [25,26], we proposed that this slow process reflects the existence of isolated quinone domains of variable PQ/PS II stoichiometries. While rapid equilibration occurs within each domain, the redistribution of quinones between

the domains occurs on a much slower time-range (30 s). This hypothesis was shown to account for the low apparent equilibrium constant. In addition, it provides a clue for another paradoxical finding concerning the oxygen-evolution yield. When photo-reduction of the pool is driven by a series of short saturating flashes, one observes the classical oscillating oxygen yield sequence, modulated by the progressive decrease due to the accumulation of $Q_A^-$ accompanying the pool reduction (low apparent equilibrium constant). However, this decrease is not accompanied by additional damping of the oscillating pattern that would be expected if each center had an increased probability of spending a greater fraction of time in the reduced state. This rather suggests that each center responds in an all-or-nothing fashion, as expected if its relationship with the locally accessible quinones is controlled by a high equilibrium constant. The lower global 'constant' which is observed must then reflect a distribution of the local PQ/center stoichiometries. As previously shown [25,26] and developed further in the companion paper [27], these features are satisfactorily explained by our model of isolated quinone domains, for which a structural interpretation, involving a network of integral membrane proteins, will be proposed. In the present paper, we wish to examine several related questions. First, how long does it take for the quinones to redistribute throughout the membrane? Second, what happens to the quinones present in unstacked regions where the density of PS II reaction centers is low? Third, what is the average number of PS II centers in the local domains? Based on this analysis, we conclude that plastoquinone cannot be involved in long distance transfer from PS II to PS I.

## Materials and Methods

*Thylakoid preparation*

Spinach or tetragone (in the experiments of Figs. 6 and 7) leaves were ground in a medium containing 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 50 mM Hepes (pH 7.5), 2 mM sodium ascorbate and 2 g/l BSA. The homogenate was filtered and centrifuged ($2000 \times g$, 10 min). The pellet was resuspended in the same medium (with no ascorbate or BSA) and kept frozen (in the presence of 5% DMSO at a chlorophyll concentration of 1–2 mg/ml) for later use.

*Measurement of absorption changes and fluorescence kinetics*

Both types of measurement were carried out in the same apparatus, previously described in Refs. 28 and 29. The principle of this apparatus is to use monochromatic microsecond flashes as a measuring light, that provides a high peak intensity (and consequently good signal-to-noise ratio) while keeping the overall illumination energy below the actinic range. The intensity of each detecting flash is measured in a reference path and used for normalizing the transmitted, or fluorescence, light signal of the the measurement path. The optical pathlength is 16 mm. When measuring ultraviolet absorption changes, the light-detecting diodes were protected from scattered actinic illumination and from fluorescence by ultraviolet-transmitting Corion SB-300F filters. Fluorescence was measured in the same geometry (180°) as absorption changes, modifying only the filter on the detecting device in the measurement path (a Wratten 96 filter was used in combination with two low fluorescence filters, Schott KV-550 and Ulano rubylith). In this mode, the excitation wavelength (detecting flashes) was set at 480 nm. The actinic continuous illumination was provided by two arrays of nine red light-emitting diodes (Toshiba TLRA-180x, peak wavelength around 660 nm) which illuminate opposite faces of the cuvette. Keeping the chlorophyll concentration low (7 μg/ml), the actinic illumination is essentially homogeneous across the sample. At maximum operating power (5 mW/diode), the intensity corresponded to about 1 absorbed photon per PS II center per millisecond. In the fluorescence measurement mode, the contribution of the fluorescence excited by the actinic beam was negligible, because of the high peak intensity of the detecting flashes and because lower frequencies are rejected electronically.

In fluorescence experiments, anaerobic conditions were obtained by adding a culture of *Escherichia coli* ($\approx 10^8$ cells/ml) to the chloroplast suspension. Incubation with the respiring bacteria was maintained for 15 min in a dark reservoir from which a sample was pumped into the measurement cuvette where it remained for 2 more min before performing the experiment. The illuminated sample was then thrown away and replaced by a fresh one. Attempts to use a glucose/glucose oxidase mixture instead of *E. coli* for achieving anaerobiosis were unsuccessful because complex quenching processes of chlorophyll fluorescence appeared under such conditions.

In ultraviolet absorption measurements, the *E. coli* method could not be used and we resorted to stirring the reservoir under an argon atmosphere for deoxygenating the suspension, a method that turned out to be somewhat less efficient. Besides, the signal-to-noise ratio for detecting absorption changes below 300 nm was found to vary largely between preparations. This is probably due to different relative concentrations of bulk proteins that both increase the absorbance background and further affect the signals, by increasing the flattening factor. With the particular batch of chloroplasts used in the fluorescence experiments, the resolution was rather poor. We could observe in this material a fast reduction of plastoquinones under saturating illumination, completed in about 100

ms, consistent with the fluorescence results, but the signal-to-noise ratio was too low for resolving the slower phase. The experiment shown in Fig. 6 was done with chloroplasts isolated from tetragone leaves that happened to yield a much better signal-to-noise performance.

*Oxygen yield measurements*

Amperometric detection of flash-induced oxygen evolution was performed using the setup described in Ref. 30. The thickness of the compartment bounded by the platinum electrode and the dialysis membrane was 0.06 mm and the chloroplasts were used at a concentration of 0.6 mg Chl/ml. The sample was placed on the electrode in the dark and dark-adapted for 10 min. During this period, an aerated buffer was allowed to flow in the upper compartment in order to fully oxidize the PQ pool. A buffer equilibrated with argon was then allowed to flow for 5 min. This flow was interrupted 2 min before starting the flashing illumination in order to complete consumption of oxygen traces by the polarized platinum electrode (at −0.7 V). We checked that a normal oxygen polarogram for photosynthetic emisssion was obtained (as previously reported [31]). We did not observe any inhibition of the oxygen evolution activity of the sample due to the polarized electrode, even after over 30 min incubation in the presence or absence of oxygen in the circulating buffer. These controls, which confirm the original work of Joliot et al. [31], are also in agreement with those described by Jursinic and Dennenberg [32] and do not substantiate the claim by Plijter et al. [33] of strong inhibition and kinetic distortion in the presence of the polarized electrode.

The time-course of the amperometric signal due to oxygen evolution upon each individual flash is mainly controlled by the time response of the transformer used as an impedance adaptor. This transient, lasting for about 1 s, was carefully determined by averaging the response in a train of low-frequency flashes. It was used to correct the data (consisting of a sampling at 8 ms after each flash) obtained at high flashing frequency (20 ms or 60 ms period) in which considerable overlap between successive flash responses occurs. A computer program was developed for that purpose.

The flash-induced oxygen yields during a series of flashes in the absence of a PS I acceptor reflect a convolution of the oscillating pattern (periodicity of four flashes) described by the Kok model [34] and of the decrease in the amount of active centers expressing accumulation of $Q_A^-$ in relation to the reduction of the PQ pool. In order to extract the amount of active centers, we designed a smoothing procedure that eliminates the Kok oscillation. The oxygen yields were first averaged over successive groups of four flashes. Plotting these values, with the first one at 2.5 flashes, the second one at 3.5 and so forth, gives a smoother sequence on which the same procedure is repeated two more times, resulting in a reasonably smooth curve starting at 5.5 flashes. This curve was extrapolated towards the first flash by fitting with an analytical sigmoid function (Figs. 8, 9). Testing this procedure on simulated sequences (with either constant or variable amount of active centers), allowed the retrieval of the $Q_A^-$ time-course within less than 1%, irrespective of the initial $S_0/S_1$ distribution adopted in the simulated sequences.

## Results

*Fluorescence induction curves*

Reduction of the PS II 'primary' acceptor $Q_A$ may be monitored through various methods such as fluorescence, oxygen evolution, C-550 absorption change or the field indicating absorption change. All these techniques give consistent results provided the non-linear relationship with the amount of active centers is taken into account [35] when using fluorescence (or oxygen-evolution rate).

The amount of electrons transfered through PS II towards secondary acceptors can then be obtained through integration of the $Q_A^-$ signal during continuous illumination of limiting intensity or during a flash series. For this integration technique to be valid, it is necessary to limit electron flow through PS I. Isolated thylakoids have lost their endogenous PS I acceptors but may transfer electrons to oxygen through the Mehler reaction. We thus used anaerobic conditions that prevent this reaction and also decrease the rate of dark reoxidation of plastoquinones. Nevertheless, as discussed below, a limited flow of electrons through PS I was still found to occur.

Fig. 1 shows anaerobic fluorescence induction curves during five successive illumination periods of 1.6 s, separated by a dark interval of 20 s. The light intensity was about 1 photon/40 ms/PS II center, which is much slower than the rate of electron transfer from $Q_A^-$ to plastoquinone. Under such conditions, the variable fluorescence is linearly related to the rate of PS II, so that the amount of electrons fed into the pool of primary and secondary acceptors can be computed from the area bounded by the fluorescence curve and the maximum level [36]. In Fig. 1 it can be seen that the maximum level has not been reached at 1.6 s during the first illumination. This is due to a slow phase lasting for about 15 s (not shown). This slow phase remains related to electron flow through PS II since it also appears in measurements of oxygen evolution under similar conditions (see Fig. 8). This suggests that part of the secondary acceptors are somehow less accessible to reduction by PS II. In principle, the slowly accessible pool fraction could be estimated by comput-

ing the area bounded by the slow phase. This method is not very accurate, since any extraneous evolution of the quenching may cause a large error. We thus resorted to the repetitive illumination procedure described below.

Upon successive illuminations the amplitude of the slow phase progressively vanishes. The area bounded by the fluorescence curve decreases until a limiting curve is reached from the fifth illumination onwards. This curve did not depend on the particular pre-illumination regime (provided sufficient illumination was given). The dark time for obtaining its complete recovery after illumination was about 2–3 s. No further modification was observed when extending this dark time up to several minutes. This suggests that a small pool of acceptors is able to reoxidize in a few seconds, while the bulk of the acceptors remain stably reduced on a minute time-range. A reoxidation in this time-range is actually expected to take place through back-reaction of $Q_A^-$ with the oxidized S-states on the PS II donor side. However, this should occur for less than one equivalent per PS II, which is not sufficient to account for the entire area of the limiting curve (about 2.5 equivalents). We propose that the additional fraction is due to a small pool of low potential acceptors associated with the PS I reaction center that are reoxidized in a few seconds in spite of the anaerobic conditions. In agreement with this view, only a smaller recovery of the area, due to $Q_A^-$ back-reaction, was observed in an algal mutant devoid of PS I centers.

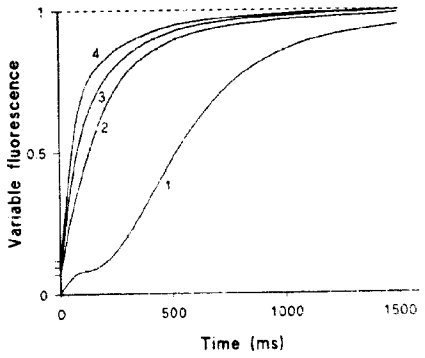


Fig. 1. Time courses of variable fluorescence under continuous illumination of limiting intensity. Thylakoids from spinach were used at a concentration of 8 $\mu$g chlorophyll per ml, in 50 mM phosphate buffer (pH 6.9), 5 mM $MgCl_2$, 1 $\mu$M gramicidin. Anaerobic conditions were achieved, as described in the Materials and Methods section. The sample was submitted to five illumination periods, separated by a dark interval of 20 s. Curve 1, dark-adapted material. Curves 2, 3, second and third illumination periods. Curve 4, limiting curve observed on the fifth illumination period (or on subsequent illuminations). The vertical scale is normalized to the maximum fluorescence yield (- - - - -). The initial level in each experiment is indicated by a horizontal segment on the ordinate scale.

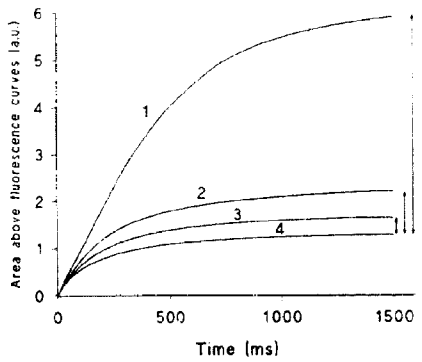


Fig. 2. Time courses of the reduction of quinone acceptors ($Q_A$ + PQ) in the experiment of Fig. 1, obtained by computing the area bounded by the fluorescence curves and the maximum level (asymptote of curve 4 in Fig. 1). The arrows show the quantities that were summed to obtain the total amount of initially oxidized acceptors, as explained in the text.

Furthermore, as documented later, no reoxidation of plastoquinol, as measured through ultraviolet absorption changes was detected within a 3 s dark period. From these considerations, one expects that the 'ever-regenerating' acceptor pool responsible for the limiting curve is involved to the same extent in each of the successive curves of Fig. 1, contributing evenly to the photo-reduction area. It should thus be subtracted in order to estimate reduction of the sole plastoquinone pool.

Fig. 2 shows a plot of the area bounded by the successive fluorescence curves of Fig. 1 as a function of time, taking the maximum fluorescence level as that of the limiting curve. According to the foregoing, in order to compute the total size of the oxidized acceptor pool ($Q_A$ + PQ) initially present under dark-adapted conditions, one should sum the contributions of each illumination, discarding the small regenerating pool present in each of them. This means adding the final levels of each curve above curve 4, as shown by the arrows in Fig. 2. As expected, this sum was found to be independent of the duration of the dark interval ($\geq$ 20 s) separating the illumination periods and of the duration of the illumination period, provided the number of cycles was sufficient to reach the steady-state. This procedure was further used for computing the data-points of Fig. 4 from the experiment of Fig. 3.

We now describe experiments aimed at studying the reduction process under high illumination intensity. This may be done, using the fluorescence technique, by separating the actinic illumination procedure from the analysis of the fluorescence area that must be obtained under limiting intensity conditions. In the experiment of Fig. 3, the chloroplasts were preilluminated by flashes of saturating intensity (about 1 photon/ms/PS

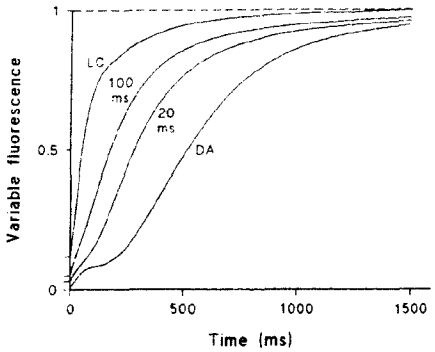


Fig. 3. Fluorescence curves under illumination of limiting intensity, in dark-adapted material (DA), or following a pre-illumination lasting 20 ms or 100 ms, with saturating continuous light. The fluorescence induction analysis was separated from the pre-illumination by a 20 s dark period. LC is the limiting curve obtained either by prolonging the duration of the pre-illumination, or by repeating light-dark cycles, as in Fig. 1. The DA and LC curves are identical to curves 1 and 4 in Fig. 1, respectively.

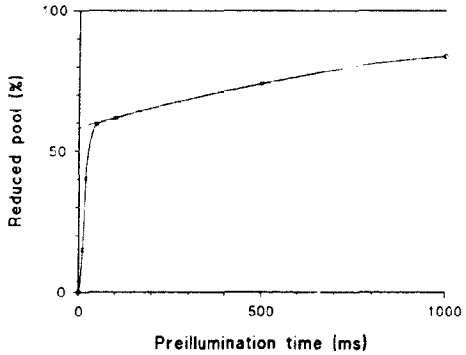


Fig. 4. Reduction of the quinone acceptors under saturating illumination (same experiment as in Fig. 3). After a pre-illumination with saturating light of variable duration (indicated on the horizontal scale), the sample was submitted to five dark-light cycles, as in the experiment of Fig. 1. The amount of oxidized acceptors was computed by summing the areas bounded by the successive fluorescence curves from which the limiting curve (LC) was subtracted (as illustrated by the arrows in Fig. 2).

II center) of variable duration. The light/dark repetitive procedure of Fig. 1 was then applied after 20 s darkness, following the high intensity flash. It is noteworthy that the various curves of Figs. 1 and 3, which all start from a low level close to $F_0$, have quite different time-courses. This illustrates an aspect of the thermodynamic paradox described in the Introduction: if quasi-equilibrium were achieved, a unique time-course should be observed (meaning that each curve should resume the dark-adapted curve from the starting point corresponding to each particular level of pool reduction), expressing a unique $Q_A/PQ$ relationship.

Fig. 4 shows a plot of the amount of remaining oxidized acceptors ($Q_A + PQ$) as a function of the duration of the saturating flash in the experiment of Fig. 3, applying the integration procedure described for Fig. 2. The reduction of the acceptor pool appears markedly biphasic with half-times of 25 ms and 800 ms. The relative amplitude of the rapid phase is here about 60% and was found to vary between 50 and 75% in different chloroplast preparations. This result shows, again, that a fraction of the plastoquinone is more slowly reduced by PS II.

As already noticed about Fig. 1, the amplitude of the slow phase in the fluorescence curve was seen to decrease in pre-illuminated samples. This decrease is also clearly observed in Fig. 3, even when the duration of the saturating flash is too short (< 100 ms) to affect the slow pool during the illumination (see Fig. 4). This suggests that an equilibration occurs between both pools during the 20 s dark period. This process was further investigated in the experiment of Fig. 5. The chloroplasts were pre-illuminated by a saturating flash of fixed duration (500 ms) amply sufficient for completing the reduction of the fast pool (see Fig. 4). A fluorescence curve was then recorded after a variable dark period and the area bounded by the fast phase of the fluorescence curve was computed. Fig. 5 is a plot of this quantity (where the asymptotic level observed for dark times ≥ 30 s was normalized to 100). As discussed above, the limiting fluorescence curve due to PS II recombination and PS I acceptors fully recovers in about 2 s: this information was used for plotting the fast phase (dashed line) in Fig. 5. The slow phase expresses the equilibration of the rapidly and slowly accessible pools, with a half-time of about 6 s.

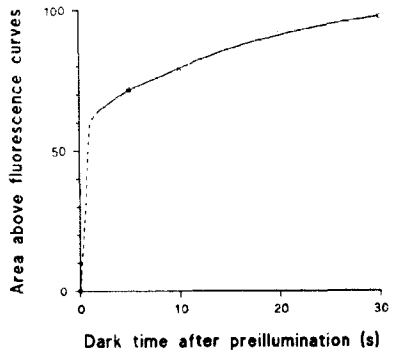


Fig. 5. Time course of the redistribution of quinones between the 'fast' and 'slow' pools. Chloroplasts were pre-illuminated by a 500 ms saturating flash and the fluorescence induction was analyzed after a variable dark period (indicated in the horizontal scale). The figure is a plot of the area bounded by the fast phase of the fluorescence curves. The dashed line was drawn by taking into account the information discussed earlier about the 2 s recovery of the limiting curve. The vertical scale was normalized by taking 100 as the asymptotic level of the fast pool area measured at times longer than 30 s.

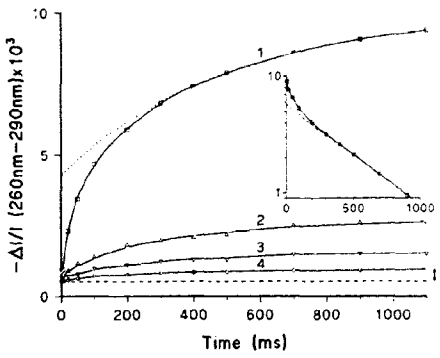


Fig. 6. Time course of the absorption change difference (260 nm–290 nm) during a saturating illumination. Tetragone chloroplasts were used at a concentration of 18 μg chlorophyll per ml in 50 mM phosphate buffer (pH 6.5), 5 mM $MgCl_2$, 1 μM gramicidin. The suspension was stirred for 15 min under a flow of argon. The samples were submitted to four light-dark cycles (1.2 s illumination, 20 s darkness). The initial millisecond-phase, with constant amplitude, was ascribed to contributions from $Q_A$ reduction and absorption changes associated with the donor side. The small PQ photo-reduction observed in the limiting curve 4 (arrow) was ascribed to reoxidation of plastoquinol during the dark period and further used in Fig. 7 (arrows in curve a). The dotted curve is an extrapolation of the slow phase, as determined in the semi-log plot shown in the inset.

*Absorption changes of plastoquinone*

In order to verify the foregoing results, reduction of plastoquinones under saturating continuous illumination was directly measured through the ultraviolet absorption change, corresponding to plastoquinol formation. Some modifications of the experimental procedure were required to obtain sufficient resolution, as explained in Materials and Methods section (using tetragone instead of spinach thylakoids and a different anaerobic treatment). The chloroplasts were submitted to cycles of saturating illumination (period 1.2 s) separated by 20 s dark intervals. At the pair of wavelengths used (260 nm/290 nm), both $Q_A^-$ and plastoquinol are detected. These contributions are however kinetically separated since total $Q_A$ reduction is completed during a fast initial phase (< 2 ms) under saturating illumination. A small contribution of changes associated with the S-state transitions is also present and mostly completed during the millisecond phase. This phase was fully regenerated during each dark period. The pool reduction following this initial rise was clearly biphasic, with a fast phase completed in about 300 ms (50 ms half-time), i.e., significantly slower than in the experiments using spinach. The inset in Fig. 6 is a semi-log plot showing kinetic discrimination between the two phases.

The total amount of reducible plastoquinone could, in principle, be directly obtained by prolonging the illumination until the slow phase is terminated. However, the measurement is then rather sensitive to baseline drift and we resorted to a summation method using successive illuminations similar to that used in fluorescence experiments. The amplitude of the slow phase progressively vanished and, from the fifth illumination on, a limiting curve (curve 4) was obtained. In contrast with the fluorescence experiments performed under strict anaerobiosis, a slow increase in the amplitude of this curve was observed when increasing the dark interval between successive illuminations, expressing reoxidation of plastoquinol through oxygen traces present in the medium. For short dark times (3 s), only $Q_A$ regenerated and plastoquinol reoxidation was negligible. Thus, the regeneration of the small pool of PS I acceptors that was inferred from fluorescence experiments does not affect the time course of plastoquinone reduction under saturating illumination. This is indeed expected since the reaction limiting overall transfer from PS II to PS I is known to be located between plastoquinol and PS I donors. The plastoquinone reduction observed in the limiting curve (curve 4) is thus entirely due to the fraction of plastoquinol being reoxidized by the oxygen of the medium during the 20 s dark period. This fraction should be approximately identical during each dark period (actually somewhat smaller during the first periods when the plastoquinol amount is smaller). To obtain the total amount of reducible plastoquinone, we thus summed the amplitudes of plastoquinone reduction from each successive curve, subtracting the fractions reoxidized during each dark period. This procedure is illustrated in Fig. 7 (curve a), where the time course during three successive illuminations was plotted, omitting the millisecond-phase. Each cycle consisted of 2 s of saturating illumination followed by 20 s of dark. The curves on the second and third illumination were positioned (as shown by the arrows) so that the first datapoint lies below the last one in the preceding curve by a distance equal to the amount of PQ reoxidation estimated from the limiting curve (arrow in Fig. 6). The horizontal line at the top shows the asymptotic level obtained by repeating this procedure, expressing the maximum amount of reducible PQ.

In Fig. 7 we further compare the photo-reduction kinetics of plastoquinones in the absence (curve a) and presence (curve b) of a sub-saturating concentration ($10^{-7}$ M) of DCMU. A biphasic photo-reduction kinetics is observed during each light period both in the absence and presence of DCMU. In the control experiment, the relative amplitude of the fast phase, with respect to the amount of oxidized plastoquinone present upon each illumination, is 0.49, 0.40 and 0.40 during, respectively, the first, second and third flashes. This recovery of the fast phase in the dark expresses the equilibration of plastoquinones between the 'fast' and 'slow' pools. In the presence of DCMU, a similar behaviour is observed, except that more cycles are

required to fully reduce the plastoquinones. The relative amplitudes of the fast phase are now 0.16, 0.17, 0.19 (with a high degree of uncertainty on the first illumination), thus decreased by about 60% with respect to the control. Correspondingly, the half-time of the fast phase is approximately doubled. The total amount of reducible PQ was, nevertheless, found to be close to that of the control (top horizontal line). In separate fluorescence experiments, we found that about 85–90% of PS II centers were inhibited in the presence of $10^{-7}$ M DCMU. Similar results (not shown) were obtained in the absence of DCMU, when partially inhibiting PS II through incubation with millimolar hydroxylamine for a short time. The finding of a decrease of the size of the 'fast' pool when inhibiting a fraction of the reaction centers is a strong indication that these quinones are contained in isolated domains (on the time-scale $< 500$ ms) including relatively small numbers of centers (see Discussion).

*Oxygen evolution*

Oxygen evolution was used as a third method for investigating electron flow towards plastoquinones. In the experiment shown in Fig. 8, dark-adapted thylakoids under strict anaerobic conditions (see Materials and Methods section), were submitted to a series of saturating short flashes, 20 ms apart. Typical oscillations with a period of four flashes were observed,

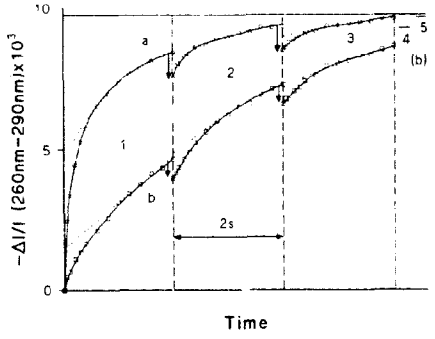


Fig. 7. Reduction of the PQ pool during three successive periods of saturating illumination (2 s) separated by 20 s darkness, in the absence (a) or presence (b) of $10^{-7}$ M DCMU. Other experimental conditions were as in Fig. 6. The initial (millisecond) phase of constant amplitude was omitted from the plot. The initial offset (arrows) for the second, third...illuminations with respect to the preceding one is the extent of PQ reoxidation during the 20 s dark interval estimated (for curve a) from curve 4 in Fig. 6. Interestingly, DCMU was found to diminish the extent of reoxidation, which accounts for the smaller offsets in curve (b). The asymptote was reached on the fourth illumination in the control, while more cycles were required in the presence of DCMU (as shown by the final levels 4 and 5 in the latter case). Identical asymptotes were obtained for the control and DCMU experiments. (· · · · · ·), extrapolations of the slow phase carried out, as shown in the inset of Fig. 6.

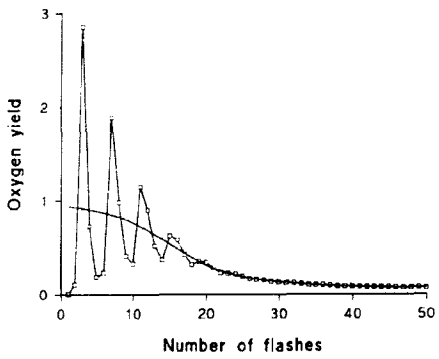


Fig. 8. Flash-induced oxygen yield during a series of saturating flashes spaced 20 ms apart. Tetragone chloroplasts in phosphate buffer (50 mM, pH 6.5), with g. micidin 0.5 $\mu$M. Anaerobic conditions were as described in the Materials and Methods section. The smooth line is the amount of open PS II centers computed, as described in the Materials and Methods section. The ordinate scale is given in electrons per center. It was computed by taking 0.94 for the initial smoothed yield ($1-\alpha+\beta$) from the miss ($\alpha = 0.08$) and double-hit ($\beta = 0.02$) probabilities estimated from the sequence.

superimposed on a progressive decrease of the oxygen evolution yield associated with reduction of the acceptor pool. This decay can be estimated (Fig. 8, solid line) by smoothing the data, as described in the Materials and Methods section. A slower phase is clearly present, similar to that observed in the fluorescence curves under continuous illumination. After several light-dark cycles, a minimum limiting rate is observed which is about 3% of the maximum initial rate. This minimum rate is not, however, the true zero of the oxygen yield, which was obtained by superimposing a strong continuous illumination on the flashes. Thus, even in the absence of oxygen, a weak steady flow through PS I still occurs, probably associated with the slow reoxidation of the small pool of low potential acceptors discussed earlier.

A noteworthy feature in the oxygen yield sequence of Fig. 8 is the persistence of the oscillating pattern when a large fraction of the centers are closed, as previously stressed [25–26]. This is a clear indication excluding the possibility of a kinetic limitation due to uncomplete turnover of the centers at this flashing frequency. If this were so, rapid damping of the sequence would occur, especially when the concentration of available oxidized secondary acceptors decreases.

In the experiment shown in Fig. 9, we investigated the effect of a partial pre-reduction of the pool on the oxygen yield sequence. The thylakoids were first pre-illuminated by a group of 15 flashes, dark-adapted for 2 min and then submitted to a series of flashes. The smoothing procedure was applied to this sequence (Fig. 9, solid line). The initial yield thus obtained was found to be about equal to the maximum yield of the

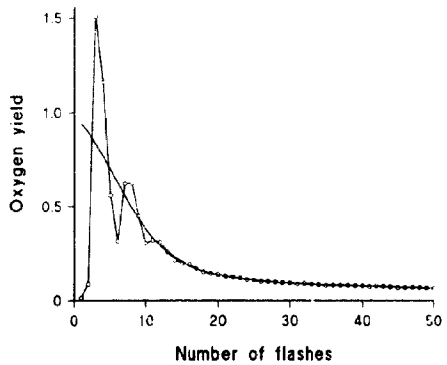


Fig. 9. Flash-induced oxygen yield in chloroplasts pre-illuminated by 15 flashes and dark-adapted for 2 mn. Other conditions as in Fig. 8.

dark-adapted sequence. By contrast, the fast phase of the decay is about twice as fast. Nevertheless, analysis of this sequence does not reveal significant additional damping compared with the dark-adapted sequence.

## Discussion

The picture that emerges from the present investigation is that of a complex compartmentation of the plastoquinone pool, in contrast with the broadly accepted view of a free diffusion of this carrier throughout the membrane. A first level of compartmentation, on a large scale, is indicated by the markedly biphasic photo-reduction of the pool. The 'fast' pool fraction represents 50–70% of the total. The stoichiometric ratio between PQ and PS II centers can be estimated from the oxygen measurements using saturating flashes. Taking into account the miss and double-hit coefficients (see the legend of Fig. 8), and subtracting the fraction of slow pool reduced during the first 50 flashes, one obtains for the fast pool, about 15 equivalents per PS II center. From this value one should subtract two to three electrons going to the regenerating PS I acceptors and also one electron for $Q_A$. Thus, the fast pool includes about six PQ molecules (each accepting two electrons) per center and the total pool 9–12 PQ per center.

The occurrence of a slow phase of photo-reduction of the pool appears in previously published material, such as the fluorescence curves shown by Forbush and Kok [22] or McCauley and Melis [4]. It may be noticed that in both these works, fresh thylakoid preparations were used, showing that the biphasic character is not specific to frozen material, as used here (an observation that we also confirmed using algal cells). McCauley and Melis used a KCN treatment in order to inhibit electron transfer to PS I through plastocyanin; this causes, as expected, a significant decrease of the fluorescence area in aerobic conditions but does not suppress the slow phase. In the Forbush and Kok paper, the photo-reduction is driven by a series of short saturating flashes, which proves that the slow phase is still observed when eliminating light harvesting efficiency (heterogeneity of the antennae) as a possible source for kinetic heterogeneity. This also applies to the oxygen-evolution experiments shown in the present paper (Figs. 8, 9) which, in addition, confirm that this phase does reflect the activity of PS II centers. It is our belief that the contribution of the slow phase was overlooked in previous estimates [2–4] of the PQ pool size, that are in good agreement with our present estimate for the fast fraction. *

The half-time for the fast pool reduction under saturating illumination was found to vary between 25 and 60 ms, depending on the material. In spinach chloroplasts (Fig. 4), the fast phase is completed in 50 ms, which means that PS II centers have turned over about twelve times during this period of time. This is clearly inconsistent with a rate-limitation of PS II expressing slow release of oxygen with $t_{1/2} \approx 30$–$130$ ms, as claimed by Plijter et al. [33]. In a recent investigation of PS II turnover rate under saturating intensity, Jursinic and Dennenberg [32] came to the same conclusion, finding a turnover time in the range 4–8 ms, in good agreement with the present estimate of about 4 ms.

A half-time of 0.8–1 s was found for the reduction of the slow pool. The experiments of Figs. 3, 5 and 7 show that both pool fractions communicate on a slow time scale. For instance, when interrupting the illumination after complete reduction of the fast pool and resuming it 20 s later, one observes a partial recovery of the fast pool – and corresponding decrease of the slow pool. This suggests a slow diffusion process between two regions of the membrane. A natural interpretation based on the structure of the thylakoid is to identify the fast and slow fractions as the PQ present in, respectively, the granal and stromal regions of the membrane. The stromal membrane accounts for about 25–35% of the membrane surface [7] and PQ has been reported to be present in the stromal membranes with a similar concentration as in granal membranes [37–38]. Estimates of the fraction of PS II centers present in the stromal region vary widely, according to organisms and methods used [39–43]. The reported density per membrane area of PS II in the grana over that in the stroma ranges from 3 [39] to 30 [43]. If we accept, anticipating the further discussion given below, that

* It may be worth noting that the previous distinction drawn by Joliot [2] or Forbush and Kok [22] between a fast ("A1") and slow ("A2") pool should not be confused with the present one: both components in the earlier work belong to the present fast pool and correspond to the kinetic break observed under saturating illumination after photo-reduction of the center-bound quinones to state $Q_A Q_B^2$.

rapid diffusion of PQ is restricted to domains of small size, photo-reduction of the stromal pool is expected to be slower because of the larger local PQ/center stoichiometry. However, it has been proposed in recent reports [44,45] that stromal PS II centers are 'inactive' with respect to PQ reduction. If this were true, the slow phase should be entirely ascribed to the diffusion of PQ from stroma to grana. This possibility, however, is not easily accomodated by our results. The photo-reduction of the slow pool under saturating illumination is a markedly faster process ($t_{1/2} \approx 0.8$–1 s) than the dark redistribution of quinones between the two regions ($t_{1/2} \approx 6$ s). Therefore, when the illumination intensity is high enough, the reduction of these quinones is not controlled primarily by their diffusion rate towards the PS II-rich granal regions. This rather supports the idea of a local photo-reduction by the fraction of PS II centers present in the stromal region. We will argue later that, when a large fraction of PS II centers is inhibited, the slow phase becomes diffusion-limited.

The difference factor of 20–30 reported here between the rates of the fast and slow phases under saturating illumination is compatible with the broad 3–30 range found in the literature for the relative density of stromal to granal PS II that should roughly reflect the PQ/PS II ratio. Nevertheless, the kinetic ratio may be larger than the relative PQ/PS II ratio, due to a lower activity of stromal centers. In agreement with these views, we found (R. Bassi and J. Lavergne, unpublished) that the majority of PS II centers in stromal membrane preparations are active, but have a diminished photochemical turnover rate.

While the biphasic reduction of the pool provides a clear indication of a large-scale heterogeneity, restricted diffusion of the quinones also appears on a finer scale, when analyzing the photo-reduction of the fast pool. As already noted, there is a dramatic discrepancy between the $Q_A$ vs. PQ relationship obtained during the photo-reduction process and the equilibrium relationship expected if full thermodynamic equilibrium were achieved. This is illustrated in Fig. 10, where the concentration of oxidized $Q_A$ was plotted against that of oxidized PQ. Curves (a) and (b) were computed from the data of Figs. 8 and 9, respectively, concerning the fast pool. Changing the flashing frequency (or light intensity in continuous illumination experiments) did not significantly modify the observed patterns. The dashed curves show theoretical equilibrium relationships for various values of the difference in mid-point potential of $Q_A$ and PQ. The S-shape expresses the fact that $Q_A$ is a one-electron carrier while PQ is a two-electron carrier. It appears that curve (a) is not only far away from the 110 mV equilibrium curve corresponding to titration data, but that it cannot be fitted with any equilibrium curve corresponding to a lower constant. As pointed out in the introduction, the two-electron gate mechanism at $Q_B$ is expected to cause some distortion to the equilibrium relationship between $Q_A$ and PQ. However, even in the odd set of states (one or three electrons on the acceptor side of the reaction center) where the equilibrium constant is lower than in the even set, the discrepancy remains obvious. This is illustrated in Fig. 10 where the open and closed circles indicate the equilibrium expected in, respectively, the even and odd states, for two values of pool reduction. By contrast, the starting point of curve b ($Q_A$ almost entirely oxidized with 50% PQ reduced), measured after a 2 min dark time, is consistent with a large equilibrium constant. As previously proposed [25,26], and discussed in the accompanying paper [27] this lack of equilibration in the second time-range is satisfactorily explained by a compartmentation of quinone domains within the granal region. A key hypothesis is that of a large heterogeneity of the PQ/PS II ratios between domains. The accumulation of $Q_A^-$ does not express a global equilibrium with the PQ pool, but rather indicates total reduction in domains where the PQ/PSII ratio is small. During the 2 min dark period following pre-illumination in the experiment of curve (b), the quinones have redistributed between domains so that global equilibrium is initially achieved.

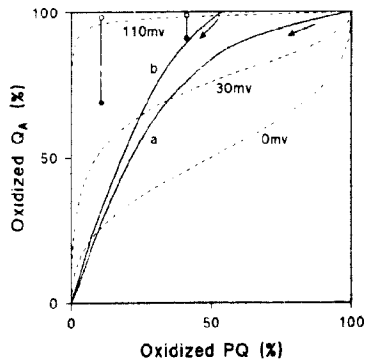


Fig. 10. A plot of the amount of open centers (oxidized $Q_A$) against the oxidized fraction of the acceptor pool, using the data of Figs. 8 (curve a) and 9 (curve b). The oxidized PQ fraction was computed by integrating the oxygen yield. The arrows show the evolution direction during the flash series (going towards total reduction of $Q_A$ and PQ). The dashed lines show the theoretical relationship for global equilibrium between $Q_A$ and PQ computed for various values of the difference ($\Delta E_m$) in mid-point potentials of $Q_A$ and PQ (as indicated): $([Q_A]/[Q_A])\cdot([PQ^{red}]/[PQ^{ox}])^{1/2} = \exp(\Delta E_m/(RT/F))$. The circles and vertical bars indicate the equilibrium range expected when taking into account the $Q_B$ gate, for two fractions of oxidized PQ (11% and 41%). The open circles correspond to the even set (see Introduction), taking $[Q_A Q_B^2]/[Q_A Q_B] = 50$ [24]. The closed circles correspond to the odd set, taking two datapoints from Fig. 3 in [24] (corrected from the 5% offset in $Q_A$ reduction observed at 100% oxidized PQ).

The experiment of Fig. 7, investigating the pool photoreduction in the presence of a sub-saturating concentration of DCMU was designed to estimate the size of the quinone domains. With $10^{-7}$ M DCMU, causing inhibition of 85–90% of PS II centers, the fast pool was decreased by about 60%. The fact that the size of the (fast) photo-reducible pool is less diminished than the number of active centers agrees with previous work showing that several PS II centers share a common pool. This conclusion was drawn by A. Joliot, investigating the effect of DCMU on oxygen evolution in algae under strong illumination [5]. The novel finding is that of a significant decrease in the fast photo-reducible pool when inhibiting a sufficient fraction of centers. Assuming homogeneous domains, each including $n$ centers, the fraction of domains where all centers are inhibited is $f^n$, where $f$ denotes the inhibition probability of one center. We obtained about 0.6 for this fraction with $f \approx 0.85$–$0.90$, which corresponds to $n \approx 3$–$4$. We do not believe the domain size to be homogeneous [26,27], so that this estimate should be taken as an approximation of the average number of centers per domain.

In this situation, where about 60% of PQ is located in 'inactive' domains in which all PS II centers are inhibited, the significance of the slow photo-reduction phase is modified. Its amplitude was found to increase, while the fast phase was diminished, so that the totality of the PQ pool remains accessible to photo-reduction. Therefore the slow phase now includes a contribution of PQ diffusion from inactive domains (most of them granal) towards neighboring active domains, where PS II centers are not all inhibited. The rate of the slow photo-reduction phase in curve (b), Fig. 7, is diminished with respect to the uninhibited curve (a), although not proportionally to the inhibition of centers (half-time about 3 s, compared to 0.9 s). Thus, this phase is now controlled by the diffusion of PQ from inactive towards active domains. It occurs with a two-fold faster rate compared to the redistribution of PQ between grana and stroma ($t_{1/2} \approx 6$ s), consistent with the smaller distances involved.

As developed in the accompanying paper [27], the structural interpretation we propose to account for the compartmentation of quinones is that the crowding of the membrane by integral protein complexes results in a network of barriers to diffusion. The free diffusion range for PQ in stacked regions involves domains containing an average of 3–4 centers. For comparison, a grana disc has a diameter of approx. 0.4 $\mu$m and the density of $EF_x$ particles is 1500–1600 $\mu m^{-2}$. Assuming that these particles are monomeric PS II centers, this means that a grana disc contains 190–200 PS II centers (twice as many if the particles turn out to be dimers). Therefore PQ diffusion from PS II to PS I involves, on average, the crossing of many domain boundaries and must be a slow process. This is consistent with the redistribution rate that we found between the fast and slow pools ($t_{1/2} \approx 6$ s), that we ascribed to diffusion of PQ between stacked and unstacked regions. An important implication is that long range quinone diffusion is too slow to be able to contribute significantly to the photosynthetic electron flow.

Clearly, our structural model is suggestive of a number of experimental tests beyond those described in the present papers. The homogeneous redistribution of membrane proteins caused by unstacking of thylakoids membranes should affect the biphasic character of the photo-reduction process; modifications of the protein density in appressed membranes through 'state-transition', by using mutant strains, or by other means, should modify the domain size. In a forthcoming paper (Joliot, P. and A. Joliot, unpublished) additional evidence supporting the domain hypothesis will be reported, based on an analysis of electron flow through the cytochrome $b_6/f$ complex.

## References


1 Whitmarsh, J. (1986) in Photosynthesis III, Encyclopedia of Plant Physiology (Staehlin, L.A. and Arntzen, C.J., eds.), Vol. 19, pp. 508–525, Springer Verlag.
2 Joliot, P. (1965) Biochim. Biophys. Acta 102, 116–134.
3 Stiehl, H.H. and Witt, H.T. (1969) Z. Naturforsch. 24, 1588–1598.
4 McCauley, S.W. and Melis, A. (1986) Photosynth. Res. 8, 3–16.
5 Joliot, A. (1968) Physiol. Vég. 6, 235–254.
6 Siggel, U., Renger, G., Stiehl, H. and Rumberg, B. (1972) Biochim. Biophys. Acta 256, 328–335.
7 Staehlin, L.A. (1986) in Photosynthesis III, Encyclopedia of Plant Physiology (Staehlin, L.A. and Arntzen, C.J. eds.), Vol. 19, pp. 1–84, Springer Verlag.
8 Albertsson, P.-Å., Andreasson, E. and Svensson, P. (1990) FEBS Lett. 273, 36–40.
9 Allred, D.R. and Staehlin, L.A. (1985) Plant Physiol. 78, 199–202.
10 Olive, J., Vallon, O., Wollman, F-A., Recouvreur, M. and Bennoun, P. (1986) Biochim. Biophys. Acta 851, 239–248.
11 Delosme, R. (1991) Photosynth. Res. 29, 45–54.
12 Haehnel, W., Ratajczac, R. and Robenek, H. (1989) J. Cell Biol. 108, 1397–1405.
13 Millner, P.A. and Barber, J. (1984) FEBS Lett. 169, 1–6.
14 Mitchell, R., Spillmann, A. and Haehnel, W. (1990) Biophys. J. 58, 1011–1024.
15 Cramer, W.A. and Butler, W.L. (1969) Biochim. Biophys. Acta 172, 503–510.
16 Knaff, D.B. (1975) FEBS Lett. 60, 331–335.
17 Horton, P. and Croze, E. (1979) Biochim. Biophys. Acta 545, 188–201.
18 Diner, B.A. and Delosme, R. (1983) Biochim. Biophys. Acta 722, 452–459.
19 Fowler, C.F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 299–307.
20 Polle, A. and Junge, W. (1986) Biochim. Biophys. Acta 848, 257–264.
21 Golbeck, J.H. and Kok, B. (1979) Biochim. Biophys. Acta 547, 347–360.
22 Forbush, B. and Kok, B. (1968) Biochim. Biophys. Acta 162, 243–253.
23 Malkin, S. (1971) Biochim. Biophys. Acta 234, 415–427.

24 Diner, B.A. (1977) Biochim. Biophys. Acta 460, 247–258.
25 Joliot, P., Lavergne, J. and Béal, D. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. 2 pp. 879–882, Kluwer, Dordrecht.
26 Lavergne, J. and Joliot, P. (1991) Trends Bio. Sci. 16, 129–134.
27 Lavergne, J., Bouchaud, J.-P., and Joliot, P. (1992) Biochim. Biophys. Acta 1101, 13–22.
28 Joliot, P., Béal, D. and Frilley, B. (1980) J. Chim. Phys. 77, 209–216.
29 Joliot, P. and Joliot, A. (1984) Biochim. Biophys. Acta 765, 210–218.
30 Joliot, P. (1965) C. R. Acad. Sci. 260, 5920–5923.
31 Joliot, P., Hofnung, M. and Chabaud, R. (1966) J. Chim. Phys. 10, 1423–1441.
32 Jursinic, P.A. and Dennenberg, R.J. (1990) Biochim. Biophys. Acta 1020, 195–206.
33 Plijter, J.J., Aalbers S.E., Barends J-P.F, Vos, M.H. and van Gorkom, H.J (1988) Biochim. Biophys. Acta 935, 299–311.
34 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
35 Joliot, A. and Joliot, P. (1964) C.R. Acad. Sci. Paris. 258, 4622–4625.
36 Malkin, S. and Kok, B. (1966) Biochim. Biophys. Acta 126, 413–432.
37 Jennings R.C., Garlaschi, F.M. and Gerola P.D. (1983) Biochim. Biophys. Acta 722, 144–149.
38 Chapman, D.J. and Barber, J. (1986) Biochim. Biophys. Acta 850, 170–172.
39 Armond, P.A. and Arntzen, C.J. (1977) Plant Physiol. 59, 398–404.
40 Andersson, B. and Haehnel, W. (1982) FEBS Lett. 146, 13–17.
41 Vallon, O., Hoyer-Hansen, G. and Simpson, D.J. (1987) Carlsberg Res. Commun. 52, 405–421.
42 Anderson, J.M. and Melis, A. (1983) Proc. Natl. Acad. Sci. USA 80, 745–759.
43 Bassi, R., Giacometti, G. and Simpson, D.J. (1988) Carlsberg Res. Commun. 53, 221–232.
44 Guenther, J.E. and Melis, A. (1990) Photosynth. Res. 23, 105–109.
45 Henrysson, T. and Sundby, C. (1990) Photosynth. Res. 25, 107–117.